The Journal of CLINICAL ENDOCRINOLOGY AND METABOLISM

Table of Contents for February 1955

Note ..... *facing page* vi

Kass, E. H.; Hechter, O.; Caspi, E. Y., and Aycock, R. R. The Route of Absorption of Orally Administered Cortisone in a Patient With Chylothorax 167

Knight, Robert P.; Kornfeld, Donald S.; Glaser, Gilbert H., and Bondy, Philip K. Effects of Intravenous Hydrocortisone on Electrolytes of Serum and Urine in Man 176

Peters, J. H.; Sieber, W. K., and Davis, N. Familial Gynecomastia Associated With Genital Abnormalities: Report of a Family 182

Green, Stanley; Evans, John M., and Hertz, Roy. Acyclic Menstrual Bleeding Associated With Erythrocytosis 199

Abramson, Daniel, and Reid, Duncan E. Use of Relaxin in Treatment of Threatened Premature Labor 206

Anker, Rudolph M. Determination of Estrogens in Stored Urines of Pregnancy .. 210

Klein, R.; Papadatos, C.; Fortunato, J., and Byers, Camilla. Acid-Hydrolyzable Corticoids of Serum 215

Goldenberg, Ira S.; Lutwak, Leo; Rosenbaum, Paul J., and Hayes, Mark A. Thyroid-Adrenocortical Metabolic Interrelations 227

Ingbar, Sidney H. Simultaneous Measurement of the Iodide-Concentrating and Protein-Binding Capacities of the Normal and Hyperfunctioning Human Thyroid Gland 238

Teaching Clinic

Kerrigan, Gerald A.; Talbot, Nathan B., and Crawford, John D. Role of the Neurohypophyseal-Antidiuretic Hormone-Renal System in Everyday Clinical Medicine 265

Editorial

Talbot, Nathan B.; Crawford, John D., and Kerrigan, Gerald. Dynamic Endocrinology in Everyday Clinical Medicine 276

The Endocrine Society

The 1955 Annual Meeting and Awards 279

The American Goiter Association

The 1955 Annual Meeting 282

The 1955 Laurentian Hormone Conference—Announcement 282

The American Geriatrics Society—1955 Annual Meeting 282

Ciba Foundation—Awards for 1954–55 in Experimental Research into Problems of Ageing 282

Books Received 283

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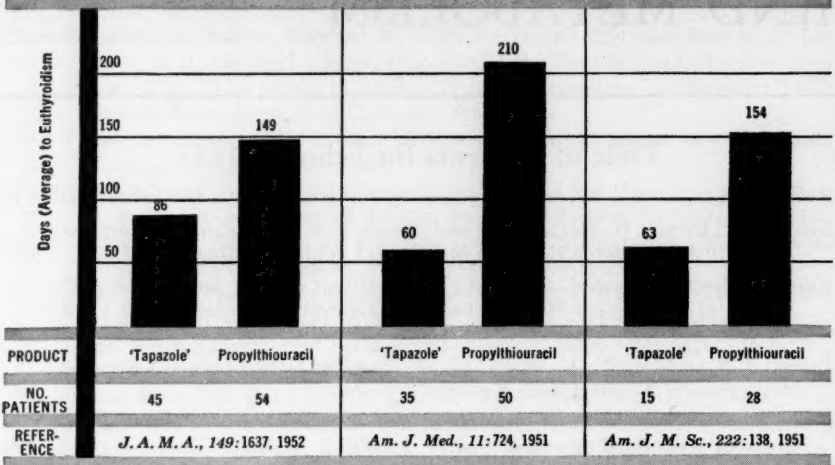

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THE ROUTE OF ABSORPTION OF ORALLY ADMINISTERED CORTISONE IN A PATIENT WITH CHYLOTHORAX*

E. H. KASS, PH.D., M.D., O. HECHTER, PH.D., E. Y. CASPI, M.PH. AND R. R. AYCOCK, M.D.

The Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital; the Department of Medicine, Harvard Medical School, Boston; and the Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts

IT IS generally agreed that such hormonal steroids as testosterone, desoxycorticosterone, progesterone, estradiol and estrone are considerably less effective by mouth than when given parenterally. The diminished effectiveness of the oral route is assumed to result from increased hepatic inactivation of these steroid hormones consequent to portal absorption from the intestinal tract. In contrast, cortisone and hydrocortisone may be administered orally with relatively little loss of anti-inflammatory activity. Two explanations for the relative effectiveness of the latter two steroids may be suggested: cortisone and hydrocortisone may be absorbed through the lymphatics, thus avoiding direct circulation of the newly absorbed hormone through the liver, or these two steroids may be absorbed through the portal system, in which case it would be necessary to assume that approximately as much hormonal activity resists hepatic inactivation as that which is available following parenteral administration of the same dose.

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Although it has generally been assumed that corticosteroids administered orally are absorbed by the portal circulation, the evidence in support of this view, although suggestive, is not direct (1). Moreover, since it has been demonstrated in rats that cholesterol and epicholesterol are absorbed through the lymphatics (2), the possibility that corticosteroids are similarly absorbed cannot be excluded.

A means for investigating the route of absorption of cortisone from the intestinal tract was presented in a patient with chylothorax (see Appendix). In this disorder, there is accumulation of chylous fluid in the thorax because of injury to the thoracic duct or a tributary lymphatic channel, so that fats taken orally appear promptly in the thoracic cavity (3). In the studies to be presented, a patient with chylothorax of unknown etiology was fed cortisone together with fat labeled with radioactive iodine. Examination of the chylous fluid revealed that, although significant amounts of the labeled fat accumulated in the thoracic cavity, no similar accumulation of cortisone occurred. Inasmuch as absorption of cortisone into the circulation could be demonstrated by a significant increase in the corticosteroid content of blood, it was inferred that cortisone was absorbed from the intestinal tract primarily through the portal circulation.

METHODS

Preparation and counting of I^{131}-labeled fat. A 5-ml. portion of peanut oil was shaken for one or two minutes with an equal volume of a solution of radioactive iodine (about 10^4 counts per second (cps)) diluted with sufficient inert carrier to impart a deep yellow color to the aqueous phase. The suspension was centrifuged to separate the two phases; the aqueous phase was colorless, demonstrating addition of iodine to the fat. The lipid phase was washed repeatedly with equal volumes of water until no radioactivity above background was detectable in the washings. Radioactivity was measured, using a heavily shielded well-type scintillation counter; 4,096 counts were recorded for each specimen and frequent background determinations were made. Volumes of biologic fluids to be counted were constant and were counted in test tubes under previously calibrated, constant geometric conditions.

Estimation of corticosteroid in chylous fluid and blood. Samples of blood and chylous fluid were extracted with ethyl acetate, using the method of Meyer (4). The ethyl acetate residues were partitioned between 80 per cent ethanol and hexane, to remove lipids. The aqueous alcoholic solution was diluted with water to reduce the concentration of ethanol to less than 10 per cent and then extracted with ethyl acetate. The residue obtained, containing the corticosteroids, was then fractionated by means of paper chromatography, by the method of Zaffaroni and Burton (5). The residues were applied to strips of washed paper and developed in the chloroform-formamide system, using both cortisone and hydrocortisone as standards on the pilot limbs. The chloroform overflows were fractionated first on toluene-formamide and then on ligroin-propylene glycol to determine whether corticosteroids less polar than cortisone, such as the C_{21} acetates of cortisone or hydrocortisone, were present. Corticosteroids were detected, using blue tetrazolium (B.T.) as the spotting reagent. The color reactions were prepared on two 0.2-cm. wide strips, cut from the sides of the chromatograms. In addition, the papergrams

were scanned with an ultraviolet lamp to detect substances possessing the Δ^4-3 ketone grouping.

Following oral administration of cortisone acetate, the only detectable corticosteroid zones observed on the papergrams of either the blood or chylous fluid represented materials of polarity equivalent to, or greater than free cortisone. No detectable amounts of corticosteroids less polar than cortisone were evident in the samples of chylous fluid or of peripheral blood. The method employed would easily have detected 5 micrograms of corticosteroid. It appears that the administered cortisone acetate is hydrolyzed in the body prior to its isolation from either blood or chylous fluid.

The corticosteroid zones detected by B.T. were eluted from the papergrams with methanol and the corticosteroid content was measured quantitatively by the method of Chen, Wheeler and Tewell (6), using both hydrocortisone and cortisone as standards. Equivalent areas of paper without B.T. reactive zones were eluted and treated to obtain a "blank" for each B.T. positive zone.

The methods employed recover 80–90 per cent of cortisone or cortisone acetate added to blood, and 67 per cent of cortisone added to chylous fluid. This latter value is lower than recoveries from blood, probably because chylous fluid contains larger amounts of fat than does blood. The values to be presented for the corticosteroid content of chylous fluid have been suitably corrected to take this factor of incomplete recovery into account.

Estimation of volume of chylous fluid. The volume of chylous fluid was estimated grossly by means of physical and radiologic examination. Attempts to use a more precise measurement of the volume of thoracic fluid by injecting known volumes of Evans Blue into the pleural space failed, because mixing of the dye was incomplete. Although the patient was asked to do such things as to roll in bed and to sit up, in order to facilitate mixing of the dye, samples of thoracic fluid removed ten minutes after the injection of dye contained concentrations of Evans Blue which indicated volumes of pleural fluid as high as 20 liters. Such impossibly high figures suggested that mixing was incomplete and the method was abandoned. Accordingly, it was necessary to use relatively crude clinical methods for the estimation of the volume of thoracic fluid. The estimate was made by two observers, using physical and radiologic evidence. It is important to emphasize that the magnitude of the results to be presented is such that it is highly unlikely that any error in estimation of pleural fluid volume would materially alter the nature of the final conclusion.

RESULTS

Accumulation of corticosteroid and fat in chylous fluid following simultaneous oral administration of cortisone acetate and I^{131}-labeled fat

About one and one-half hours after breakfast the patient was given 10 ml. of syrup of hydriodic acid by mouth in order to block thyroidal uptake of radioactive iodide. This dose was repeated twice during the next three hours. The efficacy of the blocking procedure was established by counting over the thyroid; no significant differences between the thyroidal counts and those over a nonspecific area, such as thigh, were observed. About thirty minutes after initial treatment with hydriodic acid, control samples of thoracic fluid and of venous blood were withdrawn. Immediately thereafter the patient was given 5 ml. of iodinated peanut oil con-

taining 1,070 cps of radioiodine, together with 300 mg. of cortisone acetate in the form of 25-mg. tablets by mouth. Samples of thoracic fluid and of venous blood were withdrawn one, two and five hours after oral administration of the fat and cortisone acetate. Urine was collected for seventy-two hours, to determine the extent of absorption of the fat. The total administered radioactivity was recovered in the urine—about 88 per cent in the first day, 15 per cent during the second day and 6 per cent during the third day. Virtually all of the urinary radioactivity appeared in the precipitate when silver nitrate was added to the urine, suggesting that the labeled fat had been sufficiently metabolized to permit the liberation and excretion of iodine as the iodide. It cannot be concluded, however, that the administered fat was completely absorbed from the gut, although this is

TABLE 1

Time (hrs.)	Thoracic fluid						Blood		
	Total vol. (estim'd) (ml.)	Vol. removed (ml.)	Corticosteroid (μgm./100 ml.)	Radioact. (cps./100 ml.)	Total cps.	Per cent admin'd dose (estim'd)	Vol. analyzed (ml.)	Corticosteroid (μgm./100 ml.)	Radioact. (cps./100 ml.)
0	2,000	190	26	—	—	—	25	24	0*
1	1,800	86	12	3.44	61.2	5.7	18.5	109	0
2	1,700	100	56	3.73	63.6	6.0	18	110	0
5	1,600	90	20	0.26	4.2	0.4	17	53	0

* Corresponds to background.

likely, since it is possible that some of the iodine might have been freed from the fat within the intestinal tract and subsequently absorbed.

The results obtained from the study of thoracic fluid and blood at intervals after the administration or radioactive fat and cortisone are shown in Table 1. It is convenient to discuss these results under two headings:

a. *Accumulation of radioactive iodine in chylous fluid and blood.* It is seen in Table 1 that about 6 per cent of the I^{131} that was administered as radioactive fat was present in the thoracic fluid at the end of the first hour, and 95 per cent of the radioactivity was found in the chloroform-soluble portion of the chylous fluid. The level of radioactivity in the chylous fluid was maintained during the next hour, but fell off during the next three hours. No detectable increase in radioactivity of the blood was found but it should be emphasized that the blood samples were not extracted in an attempt to concentrate small amounts of radioactivity which were undoubtedly present. The relatively small dose of tracer substance makes it unlikely that much radioactivity would be detected in the blood.

b. *Accumulation of corticosteroid in chylous fluid and blood.* Paper chromatographic examination of control samples of chylous fluid and blood showed small amounts of a single B.T. positive zone in both fluids. This

zone, of polarity approximately equivalent to corticosteroids of the O_5 series (*e.g.*, cortisone), appeared to be associated with typical responses to ultraviolet light of the Δ^4-3 ketonic steroids. Since the spot tests employed are not specific for steroids but merely measure functional groupings, the trace B.T. positive zones in the control samples were presumed to be, but not proved to be, corticosteroid. Extracts of blood obtained one and two hours after oral administration of cortisone acetate showed an intensification of the first spot and the appearance of a new, more polar zone, which had disappeared by the fifth hour. As previously mentioned, cortisone acetate could not be demonstrated by paper chromatography, using the benzene-formamide system (4).

The chylous fluid gave results similar to those on blood. Two B.T. positive zones were observed at one and two hours after feeding the cortisone, but after five hours only one zone was detected. In contrast to the reactions obtained after fractionation of blood, the zones observed in chylous fluid were small and barely detectable. Only in the sample of chylous fluid obtained at two hours after administration of cortisone was there evidence of a clear increase in concentration of corticosteroid by visual inspection. As in blood, no zone of mobility corresponding to that of cortisone acetate was detected in chylous fluid. The small amounts of material available did not permit rigorous and definitive examination of the components of the B.T. reactive zones. Instead, the entire reactive complex found on the papergrams was eluted and the total corticosteroid content measured. Since both blood and chylous fluid contained B.T. reactive substances before cortisone acetate was administered orally it was considered that any increase in total corticosteroid over control levels was due to a contribution from the orally administered steroid.

The most striking finding (Table 1) is that one hour after the oral administration of radioactive fat and cortisone acetate, significant accumulation of radioactivity was demonstrable in chylous fluid, with no increase in concentration of corticosteroid. At two hours, after the blood levels of steroid had been increased fourfold for at least one hour, there was a slight increase in the corticosteroid content of the thoracic fluid, amounting to less than 0.2 per cent of the total dose of cortisone. Total radioactivity remained at about the same level at the second hour as at the first, and returned almost to control levels by five hours, when the corticosteroid content of the chylous fluid was at control levels.

The finding that fat accumulated readily in the chylous fluid, whereas corticosteroid accumulated very little if at all, suggested that fat, but not cortisone was absorbed from the gut through the lymphatics. However, the values obtained for radioactive fat in chylous fluid may not have reflected the absorption of fat exclusively. It appeared possible that sufficient de-

iodination of the radioactive fat might have occurred in the intestines, that the iodide so liberated might have been absorbed independently of the fat, and that iodination of fat in the chyle might then have taken place. In order to study the likelihood of this occurrence, the accumulation of radioactive iodine in the chylous fluid after oral administration of potassium iodide was studied.

Accumulation in thoracic fluid of I^{131} administered orally as iodide

The patient was prepared with syrup of hydriodic acid as before and then was given orally a solution of potassium iodide registering 3,200 cps. Samples of thoracic fluid were withdrawn one and one-quarter and five hours after the administration of the radioactive iodide; the chylous fluid showed 3.1 and 4.6 cps. per 100 ml., respectively. Inasmuch as evidence of fluid was now barely detectable clinically and radiologically, and even 5-ml. samples of fluid were obtained with difficulty, it was assumed that the total volume of fluid was less than 500 ml., and that recovery of administered iodide was less than 0.5 per cent, in contrast to the 6 per cent obtained when labeled fat was fed. In this experiment, 80 per cent of the administered iodide was absorbed, as determined by counts of the urine during the next three days.

DISCUSSION

The accumulation of fat or any other substance in the chylous fluid in chylothorax represents the balance between the entrance of the substance into the thoracic space, and the exit therefrom. The accumulation of fat in the chylous fluid, established by the data presented here, confirms previous observations (3). Evidence that fat migrates readily from the thoracic cavity was obtained in an experiment in which radioactive fat was homogenized with an equal volume of chylous fluid and injected into the pleural space of the patient with chylothorax. Within three hours, 72 per cent of the radioactivity had disappeared from the chylous fluid. Similarly, protein may leave the thoracic space readily. In experiments in which Evans Blue, a dye which migrates with serum albumin in the body (7), was injected into the pleural space, it was repeatedly observed that the blue color that was imparted to the chylous fluid disappeared completely within four to twelve hours. Thus, the recovery of about 6 per cent of the ingested fat in the chylous fluid suggests that more than this actually passed through the thoracic cavity and that the persistence of this level for an additional hour reflects continued absorption of fat from the intestinal tract at a rate comparable to the rate of loss from the thoracic space.

The loss of radioactive fat from the pleural space is not due to hydrolysis of fat in the thorax with migration outward of the radioactive breakdown products of the fat. Incubation of chylous fluid containing radioactive fat

at 37° C. for twenty-four hours led to no apparent lipolysis as measured by the amount of radioactivity demonstrable in the aqueous phase after chloroform extraction of the chylous fluid.

It should not be inferred that all of the absorbed fat necessarily passes through the thoracic cavity in chylothorax. The number of tributary lymphatic channels to the thoracic duct is so great and so variable that it would be difficult to determine the percentage of the lymph that passes through the thoracic space, and how much returns directly to the venous system through collateral channels. Nevertheless, as fat is absorbed primarily through the lymphatics, and was demonstrable in significant amounts in the chylous fluid of the patient with chylothorax, the failure of fed cortisone to appear in such fluid in significant amounts indicates that the steroid was absorbed through the portal circulation.

It is noteworthy that had cortisone been absorbed by the same pathway and to the same degree as was the fat, a total of 18 milligrams would have been expected to appear in the chylous fluid, or a total increase of almost 900 micrograms in the 100-ml. aliquot of chylous fluid that was analyzed.

At the end of the second hour, a minor increase in corticosteroid concentration in the chylous fluid occurred. This increase represented about 0.2 per cent of the administered dose of cortisone, in contrast to 6 per cent of the administered dose of fat that was recovered at this time; and this increase occurred at a time when the blood levels of corticosteroid had been maintained for two hours at levels approximately twice those found in the chylous fluid.

The validity of the measurements of the radioactive fat and corticosteroid in the chylous fluid may be questioned in view of the finding that Evans Blue, injected intrapleurally, was not distributed homogeneously in the pleural fluid. However, all the Evans Blue was injected at one time, in high concentration, and a mixing period of but ten minutes was allowed. On the other hand, the fat evidently entered the pleural space at a steady rate over a period of two hours, and samples were removed at hourly intervals. The question of whether or not there was homogeneous mixing of the absorbed fat in the pleural space is, however, less critical than the finding that detectable amounts of fat were absorbed into the pleural space, whereas newly absorbed corticosteroid was not found.

The conclusion that cortisone is absorbed through the portal system presents other problems. There is a large body of evidence that hepatic tissue from several animal species transforms cortisone and hydrocortisone (8–15). Yet despite direct circulation through the liver, orally administered cortisone and hydrocortisone are approximately as active as parenterally administered hormone. This apparent paradox has been discussed previously (13) and merits further investigation.

SUMMARY

Fat, labeled with radioactive iodine, was fed to a patient with chylothorax, along with a large single dose of cortisone acetate. Significant amounts of radioactivity accumulated in the thoracic fluid, but there was no detectable accumulation of corticosteroid in the fluid at the same time. That the cortisone was absorbed was demonstrated by marked increases in blood levels of corticosteroid. The findings indicate that no significant absorption of cortisone occurred through the lymphatic pathways, hence absorption of orally administered cortisone was presumed to occur primarily through the portal venous system.

APPENDIX

Case History

G.S., a 39-year-old, unmarried laundry worker, entered the Boston City Hospital because of exertional dyspnea and dull chest pains on the right side of the chest. She had been in good health until the onset of coryza, mild frontal headaches, and a dry, nonproductive cough about five months before admission. About one month later, chest pains developed in the area of the right posterior axillary line, approximately between the fifth and eighth ribs. The pains were made worse by deep inspiration and were relieved by pressure on the affected side. About a month before admission, exertional dyspnea appeared and this became increasingly severe until the patient sought medical attention. There was no known history of trauma or severe exertion prior to admission.

There had been a 10-pound weight loss during the preceding few months. The patient's sister had been treated for tuberculosis in another city, but the patient had had no contact with her sister during the four or five years preceding treatment and not until five years after the sister's discharge from the sanatorium. The remainder of the history was not remarkable.

The pertinent physical findings were: normal temperature, pulse, and respirations, mild hypertension, and pleural effusion up to the fourth thoracic vertebra on the right. No lymphadenopathy, splenomegaly or hepatomegaly were observed.

Four urinalyses showed no abnormalities. The initial hematologic studies showed a hemoglobin of 15.0 grams per 100 ml. of blood, and a hematocrit of 47 per cent. The white blood cell count was within normal limits throughout the hospital stay, and repeated differential counts showed no abnormal cells or ratios of cellular elements. One bone marrow aspiration study showed no abnormalities. Multiple cultures of the blood and of the thoracic fluid failed to yield any growth and four different specimens of thoracic fluid and three gastric washings gave negative results for tubercle bacilli by culture and inoculation of guinea pigs. The chest fluid was opaque and milky and a creamy layer separated out on standing. Only mononuclear cells were present in the smears of the thoracic fluid, and no bacteria were seen. No malignant cells were seen in cell blocks and in direct smears.

When, as a result of repeated thoracenteses, all of the pleural fluid had been removed, and none had reaccumulated, there was no evidence by physical or roentgenologic examination of underlying pulmonary disease. The patient has been seen at three-month intervals since her discharge, has regained her lost weight, feels entirely well, and shows no physical or laboratory evidence of illness at present. The cause of the chylothorax remains obscure.

Acknowledgments

We are indebted to Dr. J. W. Strieder and the members of the Thoracic Surgical Service of the Boston City Hospital for making this patient available for study, and we are grateful to Miss Mary I. Kendrick for valuable assistance.

Addendum

The report by Bayliss *et al.* (16) appeared after this paper was submitted for publication. These workers demonstrated that orally administered cortisone was absorbed through the portal system in dogs.

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EFFECTS OF INTRAVENOUS HYDROCORTISONE ON ELECTROLYTES OF SERUM AND URINE IN MAN*

ROBERT P. KNIGHT, JR., M.D., DONALD S. KORNFELD, M.D., GILBERT H. GLASER, M.D. AND PHILIP K. BONDY, M.D.

The Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut

INTRODUCTION

THE recent introduction of hydrocortisone into clinical medicine has aroused interest in the mechanisms by which this steroid alters the electrolyte metabolism. Fourman *et al.* (1) showed that no change in the urinary excretion of sodium or chloride occurred during the first twelve hours after the intramuscular administration of hydrocortisone to normal human beings. However, two to four hours after the first injection the rate of potassium excretion began to rise. This increased rate became maximal over the next two hours and then gradually tapered off, until by the tenth hour of the study potassium was being retained. Other investigators have confirmed these results (2, 3). None of these workers described any associated changes in the serum electrolytes.

The exact manner in which the hormones of the adrenal cortex alter electrolyte physiology is not clearly understood. Investigators in this field outline, in general, three separate ways in which hydrocortisone may affect electrolyte metabolism: 1) by altering ionic exchanges across the body cell membrane, 2) by affecting electrolyte excretion in the renal tubules, and 3) through changes in protein metabolism.

The ability of adrenocortical hormones to alter the distribution of ions across the body cell membranes was first described in detail by Ferrebee and his colleagues (4). They demonstrated that about 30 per cent of the potassium in the body cells could be replaced by sodium when desoxycorticosterone acetate (DCA) was administered over a period of several days. Potassium given at the same time with DCA inhibited this effect. Although it has not yet been directly determined whether cortisone-like compounds have a similar effect, the fact that these materials cause potassium depletion and alkalosis (6) suggests that they also are capable of altering the ionic distribution.

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* This work represents a portion of the material presented by the senior author in satisfaction of the thesis requirement for the M.D. degree at the Yale University School of Medicine.

Fourman *et al.* (5) and Sprague *et al.* (6), among others, have shown that DCA and cortisone increase the excretion of potassium by increasing the renal clearance of this ion.

It is also well known that the cortisone-like hormones accelerate the rate at which protein is broken down in the body. When intracellular protein nitrogen is broken down, potassium is liberated from the cells and becomes available for excretion.

In our experiment, interest was centered on the effect on electrolyte metabolism of large amounts of hydrocortisone given intravenously over a short period of time.

METHOD

The study ran for four hours. Each subject came to the laboratory about two hours after arising in the morning, having eaten a small, low-salt breakfast. Each had voided on arising. At the laboratory a control urine specimen and blood sample were obtained. In the experimental subjects a dose of 50 mg. or 100 mg. of hydrocortisone (Merck) in a suitable carrier (5 per cent glucose and 1 per cent ethyl alcohol in distilled water, to make 250 cc. and 500 cc. of hydrocortisone solution, respectively) was infused intravenously over a four-hour period. Subjects DSK and ABC were given 50 mg.; all others received 100 mg. The subjects lay flat on a bed with the head slightly raised. Drinking water was allowed without restriction. At two hours, when half the test material had been infused, a second sample of blood and a urine specimen were obtained. At four hours, after all the material had been injected, blood and urine were again obtained, and the study was terminated.

Serum sodium and potassium concentrations were measured, using the flame photometer following the technique described by Hald (7). Serum chloride determinations were made, using Hald's micro-modification (8) of the open Carius method (9).

Eosinophils were counted according to a method described by Pilot (10), using a 1 per cent solution of phloxine as the dye.

The volume of each urine specimen was recorded and an aliquot retained for electrolyte analysis. Sodium and potassium determinations were made on the flame photometer, using the method described by Hald (7). Urine chloride determinations were performed by use of a method described by Harvey (11).

Because this study was made in conjunction with another during which serial electroencephalograms were taken, 3 of the 5 experimental subjects were well-controlled epileptics. The other 2 subjects were normal medical students. None of the 5 had any known physical abnormalities, except Subject MOR in whom a small degree of porencephaly had been demonstrated.

The 2 medical students who served as controls were given 500 cc. of the carrier solution (5 per cent glucose and 1 per cent ethyl alcohol in distilled water). The same methods were used and similar samples collected in the controls as in the experimental subjects.

RESULTS

The results are presented in detail in Table 1.

Eosinophils. In all the experimental subjects the eosinophil count dropped progressively over the four-hour period. In every instance the four-hour count was no more than one fourth of the zero-hour value. No such drop in eosinophils was observed in the control group.

TABLE 1. EFFECT OF INTRAVENOUS HYDROCORTISONE ON ELECTROLYTES OF SERUM AND URINE

Subject	Time (hrs.)	Hydrocortisone (mg.)	Eosinophils per cu. mm.	Urine Na			Urine Cl			Urine K				Serum K		Serum Cl		Serum Na	
				mEq./L.	mEq./hr.	% change (mEq./hr.)	mEq./L.	mEq./hr.	% change (mEq./hr.)	mEq./L.	mEq./hr.	% change (mEq./hr.)	Renal clearance (ml./min.)	mEq./L.	% change (mEq./L.)	mEq./L.	% change (mEq./L.)	mEq./L.	% change (mEq./L.)
RPK	0	0	28.0	114.8	9.7	—	152.0	13.0	—	49.2	4.2	—	22.6	3.10	—	104.0	—	139.0	—
	2	50	17.0	38.3	10.1	+ 4.1	55.3	14.6	+ 12.3	17.5	4.6	+ 9.5	—	3.50	+13.0	102.5	−1.4	141.0	+1.4
	4	100	<5.5	21.0	7.2	− 25.6	44.5	15.3	+ 17.7	27.2	9.3	+122.0	41.9	3.70	+19.4	100.7	−3.2	143.0	+2.8
DSK	0	0	155.0	60.0	6.0	—	67.6	6.8	—	46.4	4.6	—	23.9	3.22	—	96.5	—	137.1	—
	2	25	66.0	31.2	7.8	+ 30.0	38.3	9.6	+ 41.0	22.0	5.5	+ 19.5	—	3.50	+ 8.8	96.0	−0.5	139.8	+2.0
	4	50	22.0	11.7	6.6	+ 10.0	22.6	12.7	+ 87.0	23.2	13.0	+183.0	55.5	3.90	+21.2	97.5	+1.0	139.6	+1.8
HES	0	0	63.0	38.4	11.2	—	30.0	8.7	—	11.5	3.3	—	15.5	3.55	—	102.3	—	146.3	—
	2	50	22.0	70.6	21.2	+ 89.0	65.0	21.6	+148.0	11.4	3.4	+ 3.0	—	3.96	+11.5	101.4	−0.8	143.8	−1.7
	4	100	16.5	68.7	20.6	+ 84.0	41.0	12.3	+ 51.0	27.6	8.3	+152.0	32.2	4.30	+21.0	101.7	−0.5	145.4	−0.6
ABC	0	0	140.0	185.2	11.1	—	211.0	12.7	—	80.3	4.8	—	23.9	3.35	—	102.0	—	140.8	—
	2	25	84.0	—	—	—	—	—	—	—	—	—	—	3.53	+ 5.4	101.5	−0.4	140.6	−0.1
	4	50	35.0	173.3	27.6	+149.0	169.0	27.0	+113.0	47.3	7.6	+ 58.0	28.6	4.44	+32.0	102.3	+0.2	142.2	+1.0
MOR	0	0	29.0	87.5	14.1	—	81.7	13.5	—	19.4	3.2	—	14.2	3.75	—	96.1	—	140.0	—
	2	50	22.0	37.8	12.6	+ 10.5	37.2	12.4	+ 8.0	7.8	2.7	− 15.6	—	3.75	0.0	94.5	−1.7	141.0	+0.7
	4	100	<5.5	21.2	8.7	− 38.0	27.4	11.3	− 16.3	10.6	4.4	+ 38.0	18.3	4.00	+ 6.7	94.0	−2.2	141.5	+1.1
CONTROLS																			
RPK	0	0	66.0	207.4	12.5	—	258.0	15.5	—	79.5	4.7	—	22.1	3.55	—	99.8	—	143.5	—
	2	0	60.0	155.0	18.9	+ 50.1	162.5	19.6	+ 25.0	41.4	5.0	+ 6.4	—	4.00	+12.7	101.1	+1.5	145.3	+1.3
	4	0	71.5	34.6	12.1	− 3.2	36.9	12.6	− 17.0	13.4	4.7	0.0	20.2	3.88	+ 9.3	103.2	+2.6	140.5	−2.1
DSK	0	0	44.0	102.3	4.6	—	99.5	4.5	—	133.3	6.0	—	28.0	3.57	—	100.9	—	143.4	—
	2	0	137.0	121.6	5.5	+ 19.5	135.0	6.1	36.0	114.0	5.6	− 15.0	—	3.82	+ 7.0	100.3	−0.4	139.0	−3.1
	4	0	187.0	60.1	3.9	− 15.3	56.0	3.6	− 20.0	48.0	3.1	− 48.0	15.1	3.42	− 4.1	101.7	+1.0	140.9	−1.7

Several of the subjects showed lower initial eosinophil counts than might be expected (normal levels are usually around 150–200 cells per cu. mm.). This probably can be explained by the fact that the subjects had not been in the resting state for several hours prior to the study, and all probably had some anxiety about the experiment.

Urine electrolytes. Values were reported as milliequivalents per liter, and then converted to milliequivalents of electrolyte excreted per hour. Changes were expressed as percentage increases or decreases from the zero-hour values. The maximum analytic variation of multiple replicate analyses for each determination was as follows: urine sodium, ±6.0 mEq./L; urine chloride, ±4.0 mEq./L.; urine potassium, ±1.2 mEq./L.

In accordance with results obtained by other workers, variable and inconsistent changes were obtained over the four-hour period in regard to the excretion of sodium and chloride. Like other workers, we found a marked increase in the excretion of potassium in all 5 subjects.

Serum electrolytes. The values were reported as milliequivalents per liter. Changes were expressed as percentage alterations from the zero-hour level. The maximum analytic variation in our results was established by making repeated determinations on a single sample of serum for each ion. For serum sodium this error was ±2.0 mEq./L. (or approximately a 1.5 per cent variation for values in the normal range); for serum chloride ±1.0 mEq./L. (or 1.0 per cent); and for serum potassium ±0.3 mEq./L. (or 7.5 per cent).

We obtained results similar to those reported by other workers in regard to serum sodium and chloride concentrations, *i.e.*, neither of these ions changed consistently in any of the subjects over the four-hour period. The serum potassium level, however, showed striking changes. In 4 of the 5 subjects at the end of four hours there were rises in serum potassium level equal to from 0.6 to 1.1 mEq. The fifth subject, MOR, showed a slight rise in serum potassium concentration, which was not thought to be significant.

Although 1 control, RPK, showed a small rise in serum potassium concentration, this was not a progressive change over the four hours, nor was it of a significant magnitude. The other control showed no such change.

DISCUSSION

Whereas the changes noted in our study in regard to eosinophils and serum and urine sodium and chloride levels were no different from those previously described by other observers, the changes in potassium metabolism deserve comment. A marked increase in the excretion of potassium occurred during the four-hour infusion of hydrocortisone. In 3 subjects this increase resulted in an excretory rate substantially more than double the zero-hour level. There was a concomitant rise in the serum potassium level, an effect which has not been reported previously.

How can one best explain the simultaneous increase in the concentration

of potassium in the serum and in the urine? Fourman in his study showed that the increase in potassium excretion occurred too early to be caused by increased protein catabolism, and that the potassium diuresis was out of proportion to the nitrogen excreted. It is apparent, therefore, that the hormone probably has its early effect on the ionic exchanges which take place across the cell membrane and in the renal tubules. If the sole primary effect were on the renal tubules, one would expect either that the serum potassium concentration would fall or that the replacement of serum potassium from the intracellular fluid space would compensate for the augmented renal excretion, thus preventing any change. The present experiments, however, showed that the serum potassium concentration actually increased after hydrocortisone administration, in spite of a greatly augmented rate of excretion of this cation in the urine. It is obvious, therefore, that potassium was being released from the body cells faster than the kidney was excreting it. The reason that this effect was demonstrated in the present study whereas it was not noted in the experiments of previous workers is probably because the plasma concentration of hydrocortisone attained through intravenous administration is much greater than can be obtained by intramuscular injection. The results are even more significant when it is remembered that the glucose in the carrier for the hormone would tend to lower the serum potassium concentration. Since the renal clearance of potassium increased in each experimental subject, there was probably also a direct effect of hydrocortisone on the handling of potassium in the kidney, in addition to its effect on general cellular permeability.

No differences were noted between those subjects who received 50 mg. of hydrocortisone and those given 100 mg. This suggests that 50 mg. of hydrocortisone given intravenously over a four-hour period elicits a maximal electrolyte response.

The effects of hydrocortisone on potassium metabolism change somewhat after the first few hours. Fourman demonstrated that the maximum loss of potassium occurred four to six hours after injections were started, and that by the tenth hour potassium was being retained. The mechanism of these changes is not known, but it is possible that only a limited amount of potassium may be available for easy mobilization by the hormone, since only a part of the intracellular potassium has been shown to be freely exchangeable (12).

If hydrocortisone is given for a longer period, the serum potassium level drops below the normal concentration, where it stays as long as the administration of the hormone continues. Renal mechanisms seem to be most important here.

SUMMARY AND CONCLUSIONS

Hydrocortisone was given in large amounts intravenously over a short period of time to human beings. As expected, the blood eosinophil count

fell, and urine and serum sodium and chloride levels failed to exhibit a consistent pattern of response. With the large doses used it was also possible to show a heretofore undescribed early increase in the serum potassium level, concomitant with the expected rise in urine potassium. These changes in the serum potassium amounted to an increase of from 0.6 to 1.1 mEq. in 4 of the 5 subjects over the four hours the study was run. A fifth subject showed a smaller rise which probably was not significant.

These observations strongly suggest that one of the major early effects of hydrocortisone on electrolyte metabolism is on the distribution of cations between the intracellular and extracellular fluid. This effect in our study was so great that the increased urinary potassium excretion induced by hydrocortisone was insufficient to prevent a rise of the serum potassium concentration.

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FAMILIAL GYNECOMASTIA ASSOCIATED WITH GENITAL ABNORMALITIES: REPORT OF A FAMILY

J. H. PETERS, M.D.,* W. K. SIEBER, M.D.
AND N. DAVIS, M.D.

The Departments of Research Medicine and of Surgery, the Children's, Presbyterian and Woman's Hospitals and the Renziehausen Foundation of the University of Pittsburgh School of Medicine, Pittsburgh, and the Veterans Administration Hospital, Aspinwall, Pennsylvania

THE familial transmission of coexistent gynecomastia and hypogonadism was reported at least as early as 1893 (1). However, the importance of heredity in the transmission of male hypogonadism of various

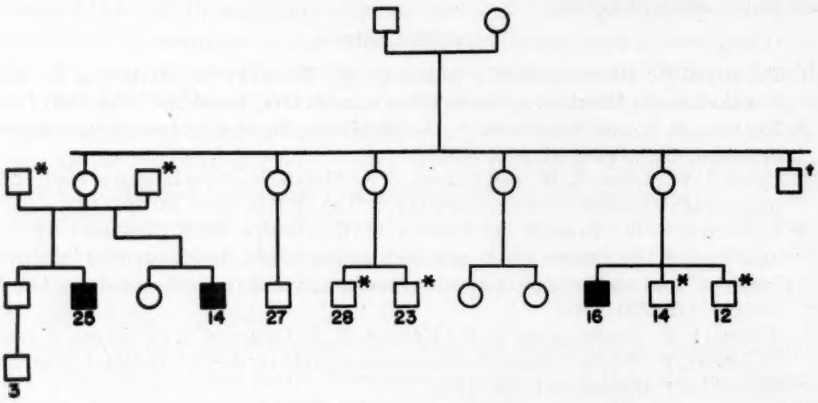

FIG. 1. Family tree. Males indicated by squares; females by circles. Solid squares indicate affected males (Cases 1–3 in order from left to right). Numbers refer to ages in 1952. Asterisks indicate definite history of normal breasts, although the 2 siblings of Patient 3 may be too young to demonstrate changes as yet. † indicates an uncle deceased in military service, thought to be normal.

types gained little recognition until recent years. Hereditary gynecomastia had been said to be "very rare" (2). A number of observations, mainly in the interval since 1940, have indicated that careful investigation may reveal familial involvement in a rather high proportion of patients with these

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* Present address: Veterans Administration Hospital, Atlanta, Georgia.

abnormalities (3–12). In 1947 Reifenstein and his associates reported a kinship of 10 males, 9 of whom had multiple abnormalities of sexual anatomy and function in which transmission appeared to be through females (6, 13). The authors' discovery of a similar family in which maternally transmitted gynecomastia, hypospadias and incomplete descent of the right testis existed in half-brothers, and gynecomastia alone in a maternal cousin, led us to examine the case records of patients with gynecomastia in three hospitals in an attempt to discover other instances of familial involvement.

MATERIALS AND METHODS

The 3 affected members of the family were observed during hospitalization for cosmetic mastectomy and the eldest was carefully questioned regarding other male members of the family. The family tree is shown in Figure 1. Creatine, serum precipitable-iodine, 17-ketosteroid and gonadotropin levels were determined by established methods (14–17).

Case 1

R.B. (#B-1472) had been admitted to Children's Hospital four times in the period 1938–40 for diagnosis and treatment of post-traumatic epilepsy. It was noted that he had hypospadias. Because the right testis was only partially descended, he was given injections of Antuitrin-S, which had no apparent effect. Marked enlargement of both breasts and a female pubic hair pattern were noted during his last admission at the age of 13.

In October 1952, aged 25, he was admitted to the Veterans Administration Hospital (#102921) for correction of his gynecomastia. He stated that his breasts had continued to grow until the age of 16, had remained large since that time and had been a frequent source of embarrassment to him. His right testis had descended intermittently into the scrotum since the age of 14. Although he had considered his penis to be smaller than average, he had had frequent erections and in recent years had had frequent intercourse with ejaculations. His beard had always been sparse and required shaving only two or three times weekly. His voice had always been rather high pitched. He stated that a younger maternal half-brother had gynecomastia and hypospadias and a maternal cousin gynecomastia alone (Fig. 1).

Physical examination. The patient had a somewhat feminine body build. His span was 67 inches; height 66 inches; and head-to-pubis and pubis-to-heel measurements 33 inches (Fig. 2B). Both breasts were diffusely enlarged and contained glandular tissue (Fig. 2A). The penis and left testis were smaller than average; the right testis was almond-sized and frequently retracted to the external inguinal ring (Fig. 2C). Urethral orifices were present at the middle and the base of the under-surface of the penis (Fig. 2D). Hair was normal in amount and texture on the extremities but sparse on the trunk. The beard was thin over the cheeks but normal on the chin and upper lip. Pubic and axillary hair was moderate in amount.

Laboratory and x-ray data. The results of serum alkaline phosphatase, cephalin flocculation, Bromsulphalein retention and prothrombin time determinations were within normal limits. The BMR was +9 per cent. The serum creatine tolerance test showed a male pattern (17). The serum precipitable-iodine level was 4.3 gamma per 100 ml. X-ray examination of the skull gave negative findings, and skeletal films showed the expected bone age.

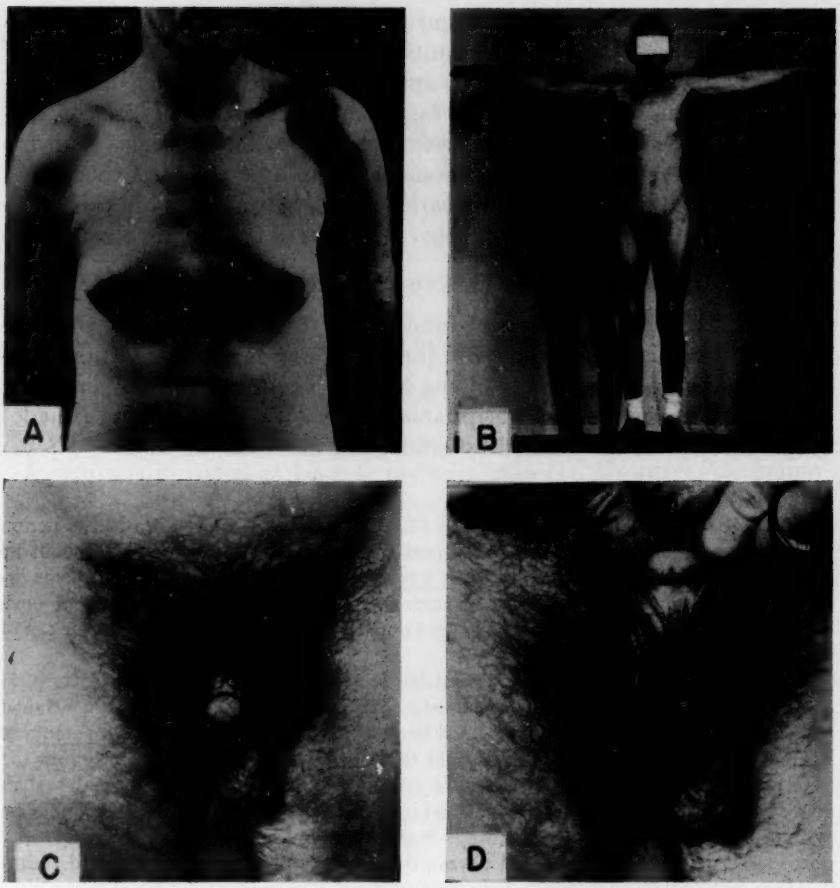

FIG. 2. Case 1. The patient prior to operation. The small right testis is in the scrotum. In D, the proximal urethral meatus is concealed by scrotal folds.

Case 2

A.L. (#Q-4466) maternal half-brother of R.B. (Case 1), entered Children's Hospital in July 1952 at the age of 14 because of enlargement of the breasts since the age of 12 (Figs. 3C and 3D). The right testis was in the inguinal canal, but descended into the scrotum intermittently. He had had a growth spurt at age 11, development of public hair at 13, and of axillary hair at 13½. He had experienced erections, but no emissions. From June to August he had received 40 mg. of testosterone per day as buccal tablets, without any detectable change in the size of the breasts. Bilateral mastectomy was performed.

In October 1952 he was readmitted for a plastic operation on the right testis, at which time hypospadias was also noted.

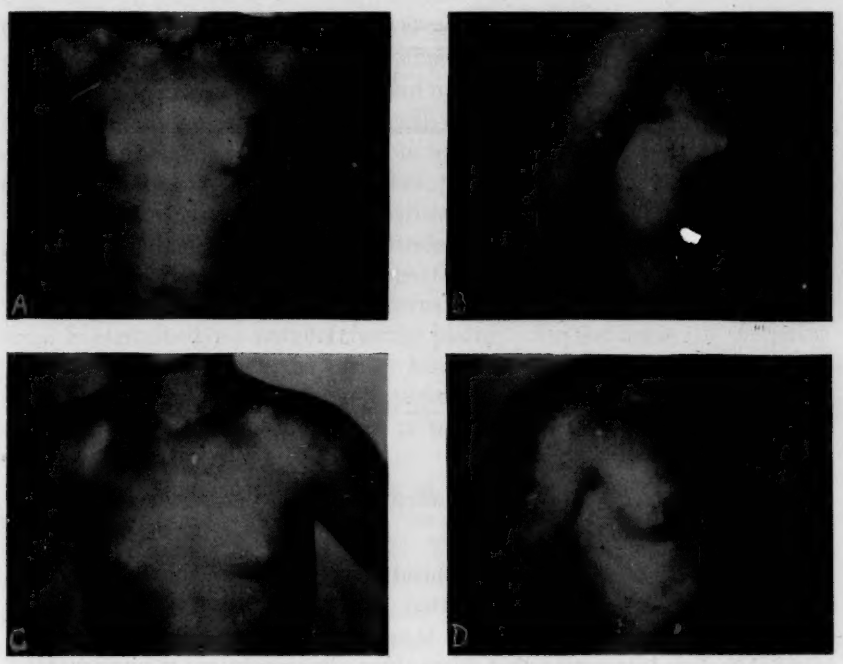

FIG. 3. Gynecomastia in Cases 2 (C and D) and 3 (A and B). (The mark on the abdomen in A is a photographic artefact.)

Case 3

C.N. (#Q-7582), 16 years old, maternal cousin of R.B. (Case 1), had noted progressive enlargement of both breasts between the ages of 12 and 14. The breasts had remained enlarged since that time (Figs. 3A and 3B). He was admitted to the hospital in September 1952 and bilateral mastectomy was performed.

Certain of the laboratory findings in these patients are presented in Table 1.

Because of our interest in this family, the records of three hospitals were examined in a search for other instances of gynecomastia. All of the case records indexed as gynecomastia between January 1, 1947 and December 30, 1952 at the Children's Hospital of Pittsburgh, the Presbyterian Hospital, and the Veterans Administration Hospital, Aspinwall, Pennsylvania, were examined. The findings are summarized in Tables 2 to 4. In addition, an attempt was made to supplement the information in the patients from Children's Hospital by sending letters to their parents requesting specific information regarding other members of the family with gynecomastia.

TABLE 1. LABORATORY FINDINGS IN FAMILIAL GYNECOMASTIA

	Date	Age (yrs.)	17-Ketosteroids (mg./24 hrs.)	Gonadotropins (m.u.u.)
Case 1	Nov., '52	25	8.5	> 6.6≤ 13
	Jan., '53			>96 <384*
	Feb., '53			>26 <106
	Apr., '53		9.9	
Case 2	1952	14	9.8	>24 < 96
	1953		11.1	
			11.9	
Case 3	1952	16	3.8	

* Courtesy of Dr. Robert Klein.

DISCUSSION

Familial transmission

Gynecomastia with associated genital defects has been shown by several observers to be maternally transmitted (6, 13, 18, 9, 10), although in many instances the mode of transmission is not indicated or is not known (7, 8, 11, 12, 19). It is interesting that when these abnormalities are unassociated with other congenital anomalies such as color blindness, females in the family are unaffected—unlike the families reported with multiple anomalies involving both sexes (4, 20, 21). The role of the mother is abundantly clear in our family, in which 2 of the patients (Cases 1 and 2), born of different husbands, had identical defects. However, male transmission has been reported (1). The female transmission of hypospadias has long been recognized (2, 22, 23) and this anomaly is apparently far more common than gynecomastia or other more extensive defects. Undescended testes in siblings (24) has also been reported as a familial defect, but the genetic origin is not known. It is of interest that although these hypogenital patterns are apparently passed through the female, male sexual precocity in families has been shown to to be carried by males (25, 26). The assumption that the fact that the right testis was the one involved in both half-brothers is further evidence of the specificity of pattern of the inherited defects seems unjustified, although it remains a possibillty.

Incidence of gynecomastia

It is difficult to determine the frequency of familial gynecomastia with or without associated hypogonadism. Certain writers make little or no mention of family history in reporting series of cases (27–36); others state that

it is uncommon (2, 37); and those interested in familial disease or in particular families say little of over-all incidence (6, 7, 8, 12, 13). Kallman, investigating the relatives of hypogonad males, found that one quarter of the cases were familial, involving one tenth of the families (4). These apparent discrepancies are in part attributable to reticence on the part of patients, and even more to the reticence of their parents and siblings who have succeeded in concealing their abnormalities. A patient reported by Witschi is a graphic illustration of this attitude (11). Furthermore, the physician frequently ignores the hereditary aspects of illnesses not usually considered "familial." A review of Tables 2–4 reveals that of a total of 75 hospital records, 8 contained no note of family history. Furthermore, in virtually every instance in which the family history was noted as being negative, the records suggested that the questions asked concerned the conventional diseases (such as diabetes, cancer, or mental illnesses) rather than gynecomastia itself.

Any discussion or interpretation of the "incidence" of gynecomastia must take into account the age of the group under consideration as well as specific etiologic factors (36, 38). The ability of many compounds—among them desoxycorticosterone (39), adrenal cortical extract (40), estrogens (41, 42, 43), methyltestosterone (44), digitalis (45), gonadotropins (46), and amphetamine (47)—to cause secondary male mammary hypertrophy has been recognized. In addition, manipulation or suckling (1, 22), hypogonadism or castration (28, 38, 48–51), familial muscle disorders (52, 53, 54), paraplegia (55, 56), adrenal tumors (51, 57, 58), testicular tumors (59, 60, 61), thyrotoxicosis (42, 62–66), and adenomas of the pituitary (3, 36) may give rise to breast development in men, and occasionally to lactation. Liver disease (42, 45, 51, 64, 67–71) and the disturbances associated with starvation and subsequent refeeding (69, 72, 73) are often associated with transient or protracted gynecomastia.

The most frequent occurrence of gynecomastia is observed during adolescence (28, 29, 32, 33, 36, 51, 74, 75, 76). The careful serial examination of a series of boys throughout puberty has revealed the phenomenon in virtually every individual (75); although as a rule the glandular changes are detected only on palpation, the swelling is transient, and the subjective phenomena are insufficient to cause families to seek medical attention (27, 28, 74, 76).

Of the 15 cases in Table 2, 10 fall into the category of "adolescent" gynecomastia, in that the duration was six months or less, and no obvious etiologic factors were noted; 1 additional case (#E-316) probably falls into the same category, despite its longer duration. If the figure of 10 per cent occurrence between the ages of 15.75 and 17.5 years in normal boys (75) is accepted, the failure to elicit a familial incidence among siblings in this

TABLE 2. GYNECOMASTIA AT CHILDREN'S HOSPITAL, 1947–52

Case No.	Age (yrs.)	Location	Duration	Family history*	Remarks
E-316	14	Lt.	1 yr.	None	"Trauma"
L-1410	14	Lt.	3 mos.	None	"Trauma"
F-4015	12	Rt.	4 mos.	None	? Precocious puberty
H-4570	7	Lt.	Life	Neg.	Phimosis, enuresis, circumcision
K-4572	14	Lt.	3 mos.	Neg.	Phimosis
L-636	14	Lt.	1+ mo.	Neg.	Rheumatic heart disease, 1947
G-2472	9	?	6 mos.	None	
J-3409	15	Bilat.	No mention	Neg.	"Mild mental retardation"
L-5337	16	Rt.	6 wks.	Neg.	Enuresis, phimosis
O-1419	14	Rt.	2 mos.	Neg.	Hydrocele, varicocele
K-6335	16	Lt.	3–5 mos.	Neg.	RHD, 1946. Had had gynecomastia on rt.
A-199	14	Rt.	4 mos.	Neg.	Tall for age
Q-4466	14	Bilat.	2 yrs.	+	Case 2
Q-7582	16	Bilat.	4 yrs.	+	Case 3
O-1449	13	Bilat.	2½ yrs.	Neg.	Jaundice at age 6

* "None" indicates no family history in hospital record.

All cases were operated upon, except #H-4570, J-3409 and #O-1449; and in all instances pathologic sections showed gynecomastia.

group must of necessity be considered to be a reflection of unsuccessful history taking. Unfortunately, the records were not complete enough to determine how many male siblings over 12 years of age were involved in the series. Undoubtedly, in a majority of instances boys take pains to conceal the changes from their associates or their parents. Furthermore, of the five families replying to our follow-up inquiries about the children listed in Table 2, two did report that other male members had had large breasts.

The operative and pathology records indicated that in the Children's Hospital group (Table 2), the hypertrophy was of the subareolar "button" type characteristic of adolescence, rather than an advanced generalized enlargement. Unfortunately, 10 of these 11 patients were operated on, 1 (#K-6335) despite the fact that previous enlargement of the contralateral breast had subsided spontaneously!

In the hospitals caring for adults, another factor must be considered, namely, that the urological services seldom index gynecomastia secondary to stilbestrol therapy for prostatic carcinoma, and the medical services usually ignore the condition when it is associated with cirrhosis. Since the individuals concerned are virtually never submitted to operation, the files of the pathology department also fail to reveal them. Therefore, although only 12 cases associated with liver dysfunction or receiving stilbestrol are

TABLE 3. GYNECOMASTIA AT PRESBYTERIAN HOSPITAL, 1947–52

Case No.	Age (yrs.)	Location	Duration	Cause	Remarks
29723	57	Lt.	7 mos.	? Liver	Epilepsy. ?Alcoholism—"5–6 beers /day"
33293	27	Bilat.	?5 yrs.	—	Refused by Navy
35684	16	Rt.	1 yr.	?	
35754	50	Rt.	2 mos.	Testicular deficiency	Bilateral testicular atrophy
37179	46	Lt.	4 mos.	?	
37910	52	Lt.	5 wks.	? Liver	Prostatic hypertrophy and cholecystitis
38167	76	Lt.	1 yr.	Liver	Tender rt. testis for 3 wks.; atrophic testes; BSP and prothromb. abnormal
38596	68	Lt.	2–3 mos.	?	Prostatic hypertrophy
38822	65	Lt.	10 wks.	?	Prostatic hypertrophy
39117	44	Lt.	5 mos.	?	Diabetes; obesity; normal serum protein and ceph. flocc.
39421	58	Rt.	2 mos.	"Trauma"	Catract. Hypertension
40859	26	Bilat.	"long hist."	—	Obesity
A-2673	31	Lt.	2 mos.	?	Multiple sclerosis
A-3353	32	Lt.	?	"Trauma"	"Lymphoblastoma" since 1944
40944	21	Rt.	"Few wks."	"Trauma"	
A-4520	74	R>Lt.	?	? Liver	Ascites; ?cirrhosis; prostatic hypertrophy
A-4702	67	Rt.	3 wks.	?	Prostatic hypertrophy
A-6065	69	Rt.	2 mos.	Liver	Prostatic hypertrophy; ceph. flocc. ++++; prothromb. 32%
A-6024	46	Lt.	1 mo.	Thyroid	BMR+32%; taking thyroid. Thyroidectomy, 1928
A-5986	47	Bilat.	1 yr.	Testicular deficiency	Mumps+testicular atrophy for 31 yrs.
A-6522	27	Bilat.	5 yrs.	Liver	Cirrhosis; hypogonadism
A-7467	28	Bilat.	4 mos.	?	
A-7514	17	Rt.	3–4 yrs.	?	
37009	69	?Bilat.	1 yr.	Estrogens	Liver disease; carcinoma of prostate; estrogen therapy
B-759	50	Lt.	2 yrs.	Thyroid	Thyroid (gr. 2 daily) for 15 yrs.
B-3486	52	Rt.	3 mos.	?	Hernia

Family histories were recorded as negative except in 4 instances in which no family history was noted in the record. All patients were operated upon except 3 (#38167, A-4520 and 37009). Surgical specimens were reported as consistent with gynecomastia except in Cases 33293 and 40859; these specimens contained only fat.

TABLE 4. GYNECOMASTIA AT ASPINWALL VETERANS ADMINISTRATION HOSPITAL, 1947–52

Case No.	Age (yrs.)	Location	Duration	Cause	Remarks
55143	65	Bilat.	?	?Testicular deficiency	Testicular atrophy. Hypertension; arteriosclerosis; heart failure; traumatic arthritis of spine. Results of liver function test normal.
56743*	24	Rt.	5–6 wks.	Tumor	Tumor of rt. breast removed in Army, 1944. Results of liver function test normal.
60266*	32	Lt.	6 wks.	?	Developed following spinal fusion operation. Removed because of pain.
60549	19	Lt.	2 yrs.	? Trauma	Developed following blow in boxing match. Operation refused.
62939	42	Lt.	10 yrs.	?	Psychoneurosis; hemorrhoids.
64898	22	Rt.	?	?	Anxiety reaction; gynecomastia incidental.
65968*	21	Rt.	?	?	Nodule removed 1945. Siladenitis.
66225	28	Lt.	8½ wks.	Thyrotoxic	Toxic diffuse goiter.
67061	25	Rt.	3 yrs.	?	Sciatic neuritis. Gynecomastia regressing.
70006*	25	Bilat.	14 yrs.	Tumor	Tuberculous tenosynovitis, nonspecific urethritis.
72279	60	Bilat.	?	?Multiple causes	Mumps orchitis as child; alcoholism; testicular atrophy; cirrhosis; beriberi.
72692*	23	Lt.	11 yrs.	?	Sudden enlargement of both breasts at 12. Rt. subsided spontaneously.
73159	55	Bilat.	?	Stilbestrol	Metast. Ca of prostate, treated by castration & orchiectomy.
73867*	24	Rt.	6 mos.	?Trauma	Bumped in 1946, with subsequent swelling and tenderness. Recurrence following another bump.
74276	51	Bilat.	?	?Liver	Mumps orchitis at 18; 3 children since; alcoholic. Results of liver function test normal.
74652	57	Bilat.	?	Stilbestrol	Ca of bladder treated with stilbestrol. Hypertension; arteriosclerosis. Previous nephrectomy.
76683	25	Bilat.	<10 mos.	?	Dermatitis, atopic.
76699	45	Bilat.	<2 yrs.	Liver	Alcoholism in past; cirrhosis; hypertension.
78197*	64	Lt.	4 mos.	?	Slight enlargement of prostate.
78212*	23	Lt.	10 mos.	?	Scoliosis.
79311	53	Bilat.	15 yrs.	?	Diabetes; arteriosclerosis. Results of liver function test normal.
79362	67	Bilat.	? 1 yr.	?	Eczematoid dermatitis; hypertension; arteriosclerosis; cataracts; arthritis.
80201	51	Bilat.	?	?	Diabetes; arteriosclerosis; hypertension.
82465*	34	Bilat.	5 mos.	?	Results of liver function test normal.
82684*	27	Rt.	1 mo.	?	Admitted for hernia.
90383*	22	Lt.	3 yrs.	?	Shell wound 3–4 mos. prior to onset.
90549*	24	Rt.	3 mos.	?	
101924	62	Bilat.	?	?Stilbestrol	Prostatectomy and castration, 1950; adrenalectomy, 1952; stilbestrol, 1950–51.
101627*	39	Bilat.	4 wks.	Thyroid Rx	Nephritis; myxedema, treated with thyroid.
101756	27	Rt.	?	?	Submucous resection.
102073	64	Lt.	?	Liver	Arteriosclerosis; obesity; cirrhosis.
102707	58	Rt.	2½ mos.	?	Atopic dermatitis; ?pernicious anemia.
102858	57	Bilat.	<2 yrs.	Castration	Castration in 1942 for Ca of prostate.
102921*	25	Bilat.	12 yrs.	Familial	See text: Case 1.
103611	56	Bilat.	?6 mos.	?	CNS syphilis; bilateral amputee; hypertension; arteriosclerosis.

* Operation. Pathologic examination showed gynecomastia except as follows: #56743, pericanalicular fibroadenoma; #70006, lipoma.

NOTE: Family histories were recorded as negative in all cases except #102921 (Case 1 in the text).

listed in Tables 3 and 4, it is known that the hospital populations during the period under discussion actually included numerous additional instances of estrogenic and hepatogenic mammary hypertrophy.

Table 5 shows that in both the veteran and nonveteran adult hospital populations approximately half of the recorded gynecomastia was of unknown etiology and that in both institutions this type was seen in about

TABLE 5. SUMMARY OF GYNECOMASTIA INCIDENCE, 1947–52

	Indexed gyneco-mastia	Total admissions	Cases per 1,000 admissions	Cases operated on	Per cent operated on
Children's Hospital	15	34,050	0.441	12	80.0
nonadolescent	5		0.147	—	—
Presbyterian Hospital	26	30,158	0.862	22	84.6
corrected*	24		0.796	20	83.3
idiopathic†	13		0.431	13	100.0
Veterans Hospital	35	45,747	0.765	14	40.0
corrected*	34		0.743	12	35.3
idiopathic†	20		0.437	10	50.0
U. S. Navy (35)			0.007–0.01‡		

* Excluding patients in whom microscopic sections failed to show gynecomastia or in whom tumor was found.

† Including cases attributed to trauma.

‡ Cases per 1,000 recruits.

1 in every 2,000 admissions. The over-all admission rate was about 1 case per thousand, reflecting far more cases of secondary gynecomastia than among the children, as would be expected from the fact that chronic hepatic disease, hormone administration, hyperthyroidism and hypogonadism are relatively infrequently encountered in pediatric practice. Although the difference between the operative rates on the veterans and on the nonveterans is striking, the origin of the discrepancy is not obvious.

Therapy

Although all of the surgical cases at Children's Hospital proved to have microscopic evidence of true gynecomastia, sections of tissue from 2 patients at Presbyterian Hospital contained only fat, and 2 patients at Aspinwall proved to have tumors, 1 a lipoma and 1 a pericanalicular fibroadenoma. This indicates first, that the degree of accuracy in preoperative diagnosis is high, and second, that the operative correction of gynecomastia in order to detect cancer is not justified, a point previously stressed by others (12, 30, 37, 72, 74). During the period 1947–52, 2 males with carcinoma of the breast were admitted to Presbyterian Hospital; both of these tumors were detected on physical examination, and neither was confused with gynecomastia. Similarly, Case #56743 in Table 4 was tabulated only because it was incorrectly indexed, since the physicians realized from the outset that the patient had a tumor, and not gynecomastia.

Psychologic hazards might be assumed to be greatest in the adolescent group, among whom the incidence is highest. The fact that such a small proportion of these individuals submitted to operation suggests that this is not the case, and this concept is strengthened by the fact that most of those who were subjected to operation had not complained of gynecomastia, but rather had been found to have it in the course of a parental or physician's examination. The individuals who display emotional aversion to mammae are largely grown men, frequently in military service (1, 22, 34, 35, 48) where nude exposure and pectoral trauma usually are unavoidable. R. B. (Case 1) commented graphically and bitterly on this point. That the stigmata which may attach to breasts are not inevitably enough of a problem to cause men to seek surgery is indicated both by the long duration noted in many of our cases (#6522, Table 3; #62939, 67061, 72692 and 79311, Table 4) and by the fact that several of these men refused operation when it was offered. Thus, Patient #A6522 entered Presbyterian Hospital solely because of the solicitude of an industrial physician and was not anxious to have an operation even then. One of us (WS) is observing a young college student who, despite marked unilateral gynecomastia, showed extreme reluctance to submit to surgery. His reluctance proved to be wise, in that recently the enlargement of his breast has almost completely regressed without therapy, after having been present for two years.

The apparently satisfactory sexual performance of R. B. (Case 1), despite the abnormalities noted, is in keeping with the findings of others (38, 48) and contrasts with the impotence seen in other types of the hypogonad syndrome. In this instance the repair of hypospadias was undertaken because of urinary dribbling. Thus, surgical or endocrine therapy may not be indicated when potency is unimpaired.

Operative or hormonal therapy of this condition should always be deferred for at least six to twelve months in the adolescent age group, and longer if the condition does not disturb the patient physically or mentally. Among adults, the presence of gynecomastia should be grounds for the initiation of a thorough investigation of nutritional status and of hepatic, adrenal and testicular function. Although testosterone propionate administration may prove effective (8, 37, 38), hormonal therapy for reduction of breast size is usually of no avail (3, 77, 78; and Cases 1 and 2), so that surgery is indicated in those instances in which the patient cannot or will not tolerate the presence of the hypertrophied organs. Using a subareolar crescent incision, removal can be accomplished without residual scars.

Laboratory findings

There is no specific pattern of hormonal imbalance characteristic of gynecomastia. In the adolescent group the excretion of estrogens or of gonado-

tropins may be increased, and 17-ketosteroids are sometimes reduced. However, none of these changes is consistently found (32). The males in the family reported by Reifenstein (6) had increased gonadotropin and normal ketosteroid excretion. Those in the family reported here had normal values for both (Table 1). The findings reported in some of the previously published series are summarized in Table 6. The relative youth of our pa-

TABLE 6. RESULTS OF URINARY HORMONE ASSAYS IN GYNECOMASTIA OF VARIOUS TYPES

Etiology	Gonadotropins	17-Ketosteroids	Estrogens	Corticoids	Pregnanediol
Dystrophia myotonia (52, 53)	=	−			
Familial eunuchs (7, 8)	−	−			
Familial eunuchs (6, 9)	+	= −			
Liver disease (64, 67)		−	+		+
Malnutrition (73, 86)	=	−	= −	=	
Paraplegia (56)		−	−	=	
Pseudohermaphrodites (18)	=	=			
Testicular atrophy (48, 50)	+	+			
Testicular failure (49, 77, 82–84)	+	= −	= −		
Testicular tumors (60, 61)	−	−	+		+
Thyrotoxicosis (64)			+		
Unknown (85)		= −	= +		

= Normal. + Increased. − Decreased or absent.

tients makes it unwise to interpret the findings with finality, although R. B. (Case 1) has reached an age when fluctuation in ketosteroids or gonadotropins might be significant, indicating that this family resembles those reported by Roth (7) and Simpson (8) rather than those observed by Reifenstein (6) and Sohval (9), in which gonadotropins were increased.

SUMMARY

A number of families have been reported in which the males had eunuchoid traits or anatomic defects of the genitalia, frequently associated with gynecomastia, which were carried through the females. The family we report is of this type. Apparently the importance of heredity in gynecomastia and male hypogonadism has been underestimated in the past. In one sense, gynecomastia is always "familial," in that it always occurs in some degree during adolescence (except apparently in hereditary precocious puberty (79, 80)), but as a rule it subsides spontaneously. Unfortunately, this common phenomenon gives rise to unnecessary fears which result in the subjec-

tion of many patients to unnecessary surgical procedures (36, 81). The paucity of available information on the natural history of breast development and function in textbooks of endocrinology is undoubtedly a factor in this attitude. Neoplasia is not a hazard; in fact, the low incidence of tumors of any sort accompanying diffuse hypertrophy of the male breast is evidence that physical examination is an adequate differential procedure.

Although one might expect that both breasts would enlarge in endocrine disorders or during hormone therapy, this is not always so. In adolescence the breasts may even enlarge at different times. Genital abnormalities of this type are not necessarily incompatible with satisfactory sexual performance, even though the individuals may be sterile. Furthermore, the presence of gynecomastia does not inevitably reflect grossly abnormal endocrine function as measured by currently available hormone assay methods, although this is frequently the case except during normal puberty.

Many patients tolerate a surprising degree of gynecomastia for long periods without seeking surgical treatment, suggesting that the emotional hazards are not insuperable, provided military or occupational situations do not give rise to trauma or embarrassment.

The discovery of gynecomastia is an indication for careful investigation of hepatic and endocrine function. Purely cosmetic surgical procedures should not be undertaken until potentially dangerous initiating processes such as thyrotoxicosis or cirrhosis have been considered.

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ACYCLIC MENSTRUAL BLEEDING ASSOCIATED WITH ERYTHROCYTOSIS

STANLEY GREEN, M.D., JOHN M. EVANS, M.D.
AND ROY HERTZ, M.D.

Endocrinoloy Branch, The National Cancer Institute, Bethesda, Maryland and The Department of Medicine, George Washington University Hospital, Washington, D. C.

EXCESSIVE acyclic menstrual bleeding associated with polycythemia is a relatively rare occurrence. It is the purpose of this paper to report such a case, secondary to congenital heart disease, and to discuss the possible mechanisms at work.

Polycythemia may occur either in response to some known stimulus, in which case it is termed erythrocytosis, or it may be of unknown etiology and then it is called erythremia (1). Both erythrocytosis and erythremia may be complicated by thromboses and hemorrhage. The latter is commonly seen in the form of epistaxis, cerebral hemorrhage, hematemesis, melena, hemoptysis and purpura.

In the course of congenital heart disease in which the contents of the right side of the heart mix with arterial blood, an increase in hematopoiesis is apt to occur. Such a situation arises in the Eisenmenger complex, the latter being characterized by (a) a membranous interventricular septal defect, (b) origin of the aorta either from both ventricles above the defect or entirely from the right ventricle, (c) right ventricular hypertrophy, and (d) absence of pulmonary or subpulmonary stenosis (2, 3). Arterial oxygen saturation is reduced in such cases and it is assumed that this low oxygen tension is a stimulus to the hematopoietic system (4).

In cases of erythremia there is an expanded red-cell mass and the circulation becomes sluggish, predisposing to thrombotic phenomena. It is thus surprising to find an increased incidence of hemorrhage in a disease so frequently characterized by thromboses (5). With the distention of the vascular tree, varices of the veins are prone to occur. The vessel walls may be weakened and they may rupture because of a disturbed blood supply. This distention of the vascular bed is apparently the most important element in the development of hemorrhages (6).

Menstrual disorders have been noted in achlorhydric anemia (7), and idiopathic thrombocytopenic purpura (8, 9), but reference to uterine bleed-

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ing in polycythemia is infrequent. Videbaek observed 125 patients, 25 to 74 years of age, having polycythemia vera for a period up to twenty years (10). There were 51 females in the group. Hemorrhage of one kind or another was found to be the most common complication in nearly three-fourths of the females, although vaginal bleeding was noted in only 1 case.

CASE REPORT

Clinical history

E.K., a 24-year-old white female, was first seen in November 1951, complaining of frequent menstrual periods.

Menarche had occurred at 12 years of age. Vaginal bleeding was variable in duration (from one to thirty-seven days) and lasted an average of nine days. During the period from November 1, 1951 to July 1, 1953 (608 days) the patient bled 44 per cent of the time (271 days) (Fig. 1). The extent of bleeding varied without pattern, from spotting to a heavy flow. There was no dysmenorrhea, and no other bleeding phenomena were noted.

She was known to have become cyanotic on occasion since birth, and to have had exertional dyspnea and easy fatigability since childhood, with aggravation following menses. She had suffered attacks of palpitation, and one such attack was followed by syncope. She had never experienced cardiac failure.

She had had measles and scarlet fever during childhood. The family history was negative for any significant illnesses. All the members of her family were relatively short. Her 2 siblings were considered to be normal and in good health. One was a sister (26 years of age) and her menstrual cycle was normal.

The patient had been working daily as a secretary. The only medication previously received was a short course of thyroid—without apparent effect. System review showed nothing abnormal.

Physical examination

The patient was a well developed, well nourished, white female having normal body proportions of normal female type. Her height was 5 feet 3 inches; weight 108 pounds; and temperature curve normal. The skin was cyanotic and minute venules were present on the eyelids, shoulders and upper anterior thorax. No abnormal pigmentations were present. There was a moderate amount of facial hair.

The eyes, ears, nose, throat and neck were normal. The breasts were full and equal bilaterally and free of masses. The lungs were clear to auscultation and normal by percussion. The respiratory rate was 20 per minute.

The heart was not enlarged to percussion. There was a regular sinus rhythm and the rate was 68 per minute. A pulmonic thrust was present without an associated thrill. The second pulmonic sound was accentuated. A soft grade II blowing diastolic murmur was heard best in the second interspace, 5 cm. to the left of the parasternal line. The blood pressure in the left arm in the sitting position was 110/70.

The findings upon abdominal, neurologic, lymphatic and rectal examinations were normal. There was no edema or clubbing and, except for cyanoses of the nail beds, there was no other abnormality of the extremities.

The external genitalia were normal. Old blood was present in the vagina. The cervix was normal in size and clear. The uterus was normal in size, contour and consistency, and was located in mid-position. The adnexae were normal. These findings were later confirmed by examination under anesthesia.

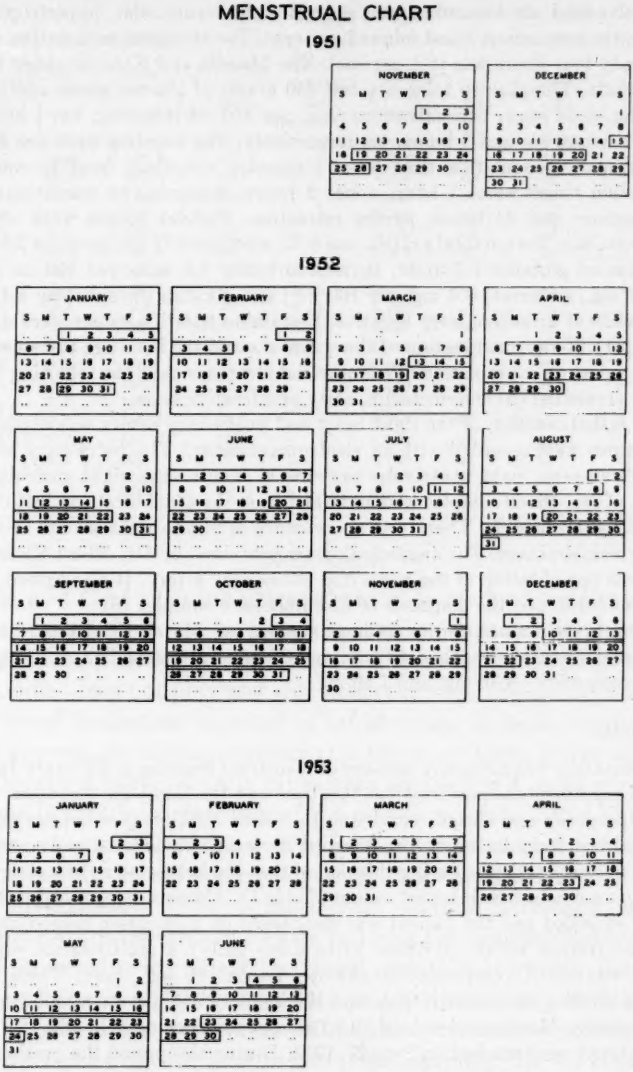

FIGURE 1

Laboratory data

Chest x-ray and fluoroscopic examination showed probable right ventricular enlargement, a normal left auricle, and enlargement of the main trunk of the pulmonary artery with slight exaggeration of the intrapulmonic vessels. There was some increase in pulmonary artery pulsations in the RAO position with barium swallow.

The twelve-lead electrocardiogram showed right ventricular hypertrophy. Basal metabolic rates were minus 3 and minus 7 per cent. The thyroidal radioactive iodine uptake in twenty-four hours was 19.1 per cent. The Massini and Kahn serologic tests gave negative results. The glucose tolerance test (50 grams of glucose given orally) showed the following blood sugar concentrations (mg. per 100 ml.): fasting, 84; ½ hour, 116; 1 hour, 120; 2 hours, 76; and 3 hours, 68, respectively. The bleeding time was 3 minutes, and the capillary coagulation time was 1½ minutes. Capillary fragility was normal. Clot retraction times were: 1 hour, none; 2 hours, beginning of retraction; 4 hours, slight retraction; and 24 hours, partial retraction. Platelet counts were 581,440 and 366,100 per cu. mm. Serum total protein was 6.75, albumin 3.78 and globulin 2.97 Gm. per 100 ml.; Kunkel globulin 7.5 units; thymol turbidity 4.4 units per 100 ml.; bilirubin (direct) 0.1 mg., (indirect) 0.4 mg. per 100 ml.; and alkaline phosphatase 4.4 units per 100 ml. Results of urinalysis were negative. Protamine titration values were within normal limits (11). X-ray examination of the wrists showed the bone age to be normal. The pathologic report on examination of endometrial scrapings gave the following diagnoses: (a) chronic cervicitis; (b) endometrium, early proliferative type.

Venous catheterization of the right heart and pulmonary artery was carried out and blood pressures were recorded with an electromanometer.[1] The pulmonary artery pressure was 87/46 mm., right ventricular pressure 87/6 mm., and right auricular pressure 3 to 6 mm. of Hg. Blood samples were obtained from the usual locations, with the exception of the right ventricle. There was no evidence of left to right shunts or anomalous pulmonary vein drainage. The angiocardiogram (obtained by Dr. Albert Kistan) showed simultaneous opacification of the aorta and pulmonary artery. It was agreed that these observations confirmed the diagnosis of Eisenmenger's complex (3).

Hormone titers (values are for 24-hour periods on March 13 and 14, 1953, respectively): gonadotropins—less than 42 m.u. and 36 m.u.; pregnanediol—0.30 mg. and 0.23 mg.; 17-ketosteroids—8.76 mg. and 7.10 mg.

Clinical course

Hospitalization was necessary because of menstrual flooding in February 1952. A dilatation and curettage was performed at this time and the histologic diagnoses were endometrial hyperplasia and chronic cervicitis. In March 1953 another hemorrhagic episode necessitated hospital admission. The bleeding decreased on bed rest and so another D & C was not necessary. It was during this period that the hemoglobin, hematocrit and prothrombin time reached their lowest values (Table 1). After six days of bed rest the bleeding finally subsided and the patient was discharged on medication consisting of protein concentrate, ferrous sulfate (0.6 Gm. three times daily), a multivitamin capsule twice daily and vitamin-K compound (Synkavite, Roche) 10 mg. daily. Table 1 indicates further the striking recuperative power of the hematopoietic system following the hemorrhagic episode. Maximum levels of 19.3 Gm. per cent hemoglobin and 76 volumes per cent hematocrit were reached on June 27, 1953. During this period the prothrombin time did rise to more normal levels but the menstrual pattern continued as it had in the past.

An additional ninety days' observation of this patient (from July 1, 1953 to November 30, 1953) revealed an acyclic pattern of menstrual bleeding much like that illustrated in Figure 1. The combined data indicate that during the period from November 1, 1951 to November 30, 1953 (761 days) the patient bled 47 per cent of the time (361 days).

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[1] Sanborn Company, Cambridge, Mass.

TABLE 1

Date	Hb. (Gm./100 ml.)	Hb. (% of normal)	Hemato-crit (vol. %)	RBC (million per cu./mm.)	WBC (per cu. mm.)	Poly. (%)	Lymph. (%)	Eos. (%)	Pro-thrombin (% of normal)
Nov. 27, '51	18.2	121.3	65		6,050	46	53	1	71
Dec. 6, '51	18.2	121.3	66		8,100	58	40	2	
Feb. 8, '52	16.9	112.6	62		9,400	54	46		
Feb. 18, '52	15.6	104	56		9,700	54	46		
Feb. 25, '53	15.6	104	60		9,750	54	46		
Mar. 10, '53	13.4	89.3	51		9,600	57	41	2	
Mar. 11, '53	12.4	82.6	47	4.45					45
Mar. 12, '53	12.2	81.3	47	4.24	9,300	51	45	4	
Mar. 13, '53	12.4	82.6	46	5.23	8,650	66	33	1	35
Mar. 14, '53	12.4	82.6	45	5.1	8,650	66	33	1	45
Mar. 16, '53	11.8	78.9	45	4.81	8,650	34	55	1	75
Mar. 24, '53	14.8	98.6	51		9,000	59	40		
Apr. 4, '53	16.0	106.6	62		6,950	52	48		61
Apr. 14, '53	16.0	106.6	66		7,300	49	49	2	40
May 2, '53	17.3	115.3	66		6,250	62	34	4	50
May 16, '53	18.2	121.3	68		9,550	67	33		75
June 5, '53	18.2	121.3	73		9,100	56	42	2	72
June 27, '53	19.3	128.6	76		10,400	58	41	1	72
Nov. 12, '53	18.2	121.3	77	8.30	8,250	52	48		

DISCUSSION

Haynal and Graf (12) investigated the role of the hypophyseal-hypothalamic area of the brain in producing both symptomatic polycythemia and erythremia. Although oxygen lack, the stimulating factor in secondary polycythemia, does not play any role in erythremia, the pathogenesis of increased blood formation appears to be identical in both conditions. By means of glucose, insulin and epinephrine tolerance tests, it was determined in 8 of 10 cases of polycythemia rubra vera and in 3 of 4 cases of secondary polycythemia that hyperfunction of the pituitary existed. In 1 of the latter cases, there was clinical evidence of hyperfunction, *i.e.*, precocious puberty with menstruation in the absence of other endocrinopathy.

These investigators demonstrated that steroid hormones which depressed pituitary function sufficiently to bring the tolerance curves to normal did not significantly affect the blood count. It is known that tumors in the hypothalamic region will produce polycythemia (12), and that hypophysectomy will produce a decrease in reticulocytes and normoblasts which can be corrected by injections of hypophyseal extracts (13, 4). It is felt that the anemia which follows hypophysectomy is not due to the loss of any specific hemopoietic factor. Hematopoiesis is not primarily under endocrine control. Rather, the general upset of the metabolism of the tissues (including the bone marrow) following loss of the pituitary accounts for the profound effect on the hemogram (14, 15, 16).

Based upon an evaluation of carbohydrate metabolism, thyroid and adrenal function, gonadotropin levels and x-ray examination of the skull,

there was no evidence of pituitary abnormality in the patient described in our report. It is quite possible, however, that such an abnormality might be expressed solely as an acyclic menstrual pattern. Confirmation of such a supposition must await finer techniques for measuring pituitary secretions and ovarian function.

There was no indication of any abnormality in the coagulation mechanism in this case, except for the lowering of the prothrombin below 50 per cent on several occasions during the period of maximum bleeding. The menometrorrhagia was, however, also found in association with more nearly normal prothrombin values. There was no evidence of hemorrhage from sites other than the uterus. Histologically there was no apparent abnormality of the endometrial vessels which might predispose to their rupture.

The etiology of acyclic menstrual bleeding is not known but, theoretically, abnormal function of the pituitary or failure of the ovary to respond properly to the pituitary could upset the normal balance. It seems most likely that the vaginal bleeding in this case was due to hormonal imbalance rather than to overdistention of the endometrial vessels. Statistically, it would seem that the endometrial vessels are particularly resistant to the factors which cause hemorrhage in polycythemia. The postulated endocrinopathy might well have been coincidental to the polycythemia, or it might possibly have been due to hyperfunction of the hypophyseal-hypothalamic system, a condition which has been shown to occur in polycythemia of both types (12).

The patient's work-tolerance was much higher when supplemental medication aimed at increasing the blood count was given. The question arises as to what hemoglobin value is optimal and whether the vaginal bleeding acted as a compensatory measure in preventing the thrombotic complications of polycythemia, in much the same manner as repeated venesection. If such be the case, then in recommending hysterectomy or hormonal depresssion of acyclic bleeding one must consider the possibility that inability to lose excess blood volume might predispose to thrombotic or hemorrhagic phenomena in other areas of the body.

The concept of hyperpituitarism secondary to anoxemia acting as the direct stimulant to the bone marrow and producing uninterrupted stimulation of the ovary resulting in acyclic menstrual bleeding is very intriguing, particularly since no other evidence of increased function of the pituitary was determined. Such a pattern, although not established at this time, could explain all of the features of this reported case.

SUMMARY

A case is reported of acyclic menstrual bleeding associated with erythrocytosis secondary to congenital heart disease in a 24-year-old female.

During an observed period of 761 days, the patient bled 47 per cent of the time (361 days).

Statistically, uterine bleeding is very infrequent in polycythemia in comparison to hemorrhage from other sites. In the patient concerned, there was no evidence of bleeding from other sites of the body and no defect in the coagulatory mechanism. Histologically, there was no apparent abnormality of the endometrial vessels.

The clinical course was complicated by an episode of uterine hemorrhage and the amazing recuperative power of the hematopoietic system was revealed.

The possible hypophyseal-hypothalamic mechanism at work in producing this complex is discussed.

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USE OF RELAXIN IN TREATMENT OF THREATENED PREMATURE LABOR

DANIEL ABRAMSON, M.D. AND DUNCAN E. REID, M.D.

The Boston Lying-in Hospital and the Department of Obstetrics, Harvard Medical School, Boston, Massachusetts

SINCE the discovery of the hormone, relaxin, by Hisaw in 1926 (1) as a result of its ability to cause pelvic relaxation of the estrogen-primed guinea pig, numerous reports of the effect of this substance in animal experimentation have appeared in the literature (2, 3, 4). Species differences in its action have been observed (5–9), however, and consequently, it is difficult to deduce from animal experimentation what its possible effect might be in the human being.

Relaxin has been recovered from human pregnancy serum in the first trimester, as reported by Abramson *et al.* (10, 11). Recently Zarrow (12) has found relaxin in human serum throughout pregnancy. The function of this hormone in the human female remains to be defined. Some degree of relaxation of the pelvic joints is accepted as one of the changes in normal pregnancy (13). Sawyer *et al.* (14) have demonstrated complete inhibition of spontaneous contractions of rat uteri with relaxin *in vitro*, using extremely small doses. Whether relaxin has the property to restrain contractility of the human myometrium and thereby influence uterine motility in human pregnancy or to cause anatomic alterations of the uterus *in vivo* remains to be established.

Clinical experience attests to the unsatisfactory results in the treatment of premature labor by the sex steroids, singly or in various combinations. Recently a potent product of relaxin has become available for clinical use. Studies on humans by the authors (15) and by Perkoff *et al.* (16) dispel fear of toxicity of this commercial preparation, even in very high dosage.

The purpose of this communication is to record our clinical experience with the use of this preparation in the treatment of threatened premature labor. The data consist of the observations in only 5 patients[1] and are therefore presented as a preliminary report.

CLINICAL OBSERVATIONS

The substance used in these patients was a commerical preparation of relaxin, called Releasin.[2] It was given intramuscularly in dosages of from

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[1] Since submission of this manuscript for publication, the number of patients studied has increased to 25.

[2] Releasin was prepared and kindly supplied by the Warner-Chilcott Laboratories.

2,000 to 5,000 guinea-pig units (17), repeated as indicated. The following case reports are given in detail:

Case No. 1.

Mrs. J. K., a 26-year-old gravida i, para 0, entered the hospital in her thirtieth week of gestation, with a history of abdominal pain and vomiting of three days' duration. On the day after admission an appendectomy and drainage of an appendiceal abscess was performed. Convalescence was essentially uneventful until the seventh postoperative day, when painful uterine contractions occurred which were not influenced by daily administration of stilbestrol (50 mg. three times daily) and progesterone (50 mg. twice daily). Morphine sulphate in repeated doses failed to eliminate contractions, which continued every two to three minutes, and were of thirty to sixty seconds' duration. Twelve hours after onset of these contractions Releasin, in a dose of 2,000 units, was given intramuscularly and repeated in two hours. One hour after the first injection the contractions decreased to thirty seconds' duration, and shortly after the second dose they ceased entirely. The patient was discharged six days later, improved. She was delivered of a normal infant in the thirty-ninth week of her pregnancy.

Case No. 2.

Mrs. N. K., a 24-year-old gravida i, para 0, with an uncomplicated prenatal course, entered the hospital in her thirtieth week of gestation, with painful uterine contractions. On admission she was noted to have contractions every three minutes, lasting thirty seconds. The cervix was dilated 1 cm. The patient was given 100 mg. of Demerol without relief, and two hours later her pains were occurring every three minutes and were of forty seconds' duration. There was moderate bloody discharge from the vagina. The uterine pains increased in intensity and frequency. At this time the patient was given 2,000 units of Releasin intramuscularly; this dose was repeated in two hours, following which she experienced only an occasional contraction. Although the pains disappeared, the uterus did contract infrequently. For this reason the patient was given two more injections of Releasin (2,000 units each) the following day. Labor having completely ceased, the patient was discharged that evening. At thirty-nine weeks of gestation, following seven hours of labor, the patient was delivered by low forceps of a normal infant.

Case No. 3.

Mrs. M. N., a 37-year-old gravida iii, para ii, with an uncomplicated prenatal course, entered the hospital in her twenty-ninth week of gestation having painful uterine contractions every two to five minutes, lasting thirty seconds. One hour after admission she was given 4,000 units of Releasin intramuscularly, followed in one hour by 2,000 units intramuscularly. Contractions of the uterus slowed to every two to seven minutes, of only ten to fifteen seconds' duration. One more injection of 2,000 units was given three and one-half hours after the last dose, as a prophylactic measure. The patient was discharged the following day, undelivered, and having no contractions. She was delivered by low forceps of a normal infant, at term, after ten hours of labor. Because of Rh sensitivity in the mother, the baby was given an exsanguination transfusion.

Case No. 4.

Mrs. D. M., a 31-year-old gravida iii, para ii, entered the hospital in her thirty-third week of gestation, having painful uterine contractions every three to four minutes, lasting thirty to fifty seconds. One and one-half hours later she was given 5,000 units of Re-

leasin intramuscularly, and this dose was repeated every half hour for five doses. Three hours after the first injection of Releasin occasional contractions were noted, lasting twenty to thirty seconds. During the ensuing thirteen hours, because of occasional contractions and moderate bloody vaginal discharge, three more injections of Releasin (5,000 units each) were given. Following the last injection no further contractions occurred, and the patient was discharged twenty-four hours later, undelivered. She was delivered in her thirty-sixth week of gestation, after two hours of labor, of a normal infant.

Case No. 5.

Mrs. S. F., a 31-year-old gravida vi, para ii, entered the hospital in her thirty-second week of gestation, with recent rupture of the membranes. Approximately fifteen hours later she began to have a few uterine contractions. At this time she received 5,000 units of Releasin intramuscularly, and the dose was repeated two hours later. She received four injections of 5,000 units each during the next four hours, after which her contractions subsided. During the ensuing nine hours, because of irregular contractions, she was given six injections of 5,000 units each at intervals at one-half to three hours, following which her contractions ceased entirely. The patient was discharged one week after admission in her thirty-third week of gestation, not in labor but continuing to lose a small amount of amniotic fluid. She was delivered in her thirty-seventh week of gestation, after three hours of labor, of a normal infant.

SUMMARY

In 5 patients who entered the hospital in premature labor, between the twenty-ninth and thirty-first weeks of pregnancy, labor ceased following administration of relaxin intramuscularly. Pregnancy in each case continued through the thirty-sixth to fortieth weeks, with the delivery of a normal infant in each instance.

Though the group of patients reported constitutes a small series, the uniformity of action of relaxin in each case is noteworthy. If future observations prove comparable, the incidence of premature labor and its attendant fetal mortality could be decreased.

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DETERMINATION OF ESTROGENS IN STORED URINES OF PREGNANCY*

RUDOLPH M. ANKER, PH.D.

Endocrinology Laboratory, Department of Obstetrics and Gynecology, University of Colorado School of Medicine, Denver, Colorado

THE results reported in this communication were obtained in the course of a study requiring the determination of estrogens and pregnanediol in a large number of urine samples which had been stored for a long period of time. The determination of estrogens in human pregnancy urine has to to be preceded by the complete cleavage of all estrogen conjugates in such urines, and this should be accomplished without any destruction of the liberated estrogenic steroids if these determinations are to be truly quantitative. A method satisfying these requirements has not been described as yet (1). Therefore an attempt has been made to ascertain the conditions for optimal recovery of estrogens from their conjugates; and the subsequent extraction and concentration procedure has been simplified to the greatest extent consistent with a quantitative recovery of the deconjugated estrogens. The estrogens were determined by a modification of the Kober test (2) described by Allen (3), and the conditions for maximal efficiency of this test were ascertained.

METHODS

The urine specimens were stored at room temperature covered with a layer of *n*-butanol.

Recovery of estrogens from human pregnancy urine: The method worked out here combines the extraction procedure of Engel *et al.* (4), which is based upon the results of their partition experiments, with the less destructive and more convenient hydrolysis of Dingemanse *et al.* (5). In addition, the method was shortened by the elimination of all avoidable manipulations.

A suitable aliquot of the urine specimen is boiled to expel the butanol, the condenser is set for reflux, toluene (20 per cent by volume) and strong hydrochloric acid (15 per cent by volume) are added carefully through the condenser and boiling is continued for 90 minutes. The cooled solution is filtered and the filter rinsed with water and hot toluene. The toluene layer is separated and set aside. The aqueous layer is extracted once

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with one-half volume and three times with one-quarter volume of peroxide-free ether. The combined ether extracts are evaporated, and the toluene layer is added to the residue. The toluene is extracted four times with one-quarter volume of 1N sodium hydroxide. That part of the residue which is not soluble in toluene should be thoroughly exposed to the alkali also. The toluene layer may now be used for the assay of pregnanediol (6) The alkaline extracts are acidified with a slight excess of strong hydrochloric acid as soon as possible and the combined acidified extracts are brought to pH 8.5 to 9.0 by the addition of a saturated solution of sodium carbonate. The solution is extracted once with one-half volume and three times with one-quarter volume of peroxide-free ether. The combined ether extracts are washed with one-twentieth volume of water, and the aqueous layers are discarded. The ether extracts are evaporated and the residue taken up in a suitable volume of absolute ethanol for colorimetric assay. The acidified aqueous extracts form a deposit on standing over night, even at pH 9. This deposit occludes estrogens which cannot be recovered by extraction with ether; therefore the steps starting from the extractions with alkali should be carried out during one working period.

If the ether extractions in this scheme are reduced from four to two and the toluene layer is extracted only twice with one-half volume of 1N sodium hydroxide, 95 per cent of the unconjugated estrogens are recovered. This is sufficient for many purposes and reduces the amount of time and labor required.

The colorimetric assay of estrogens: Aliquots of extracts containing 15 to 70 μg. of estrogens are pipetted into test tubes and evaporated to dryness by placing the tubes in a bath of warm water and passing a very gentle current of air. Errors are introduced very easily at this stage; they are not affected by the use of nitrogen, air or oxygen for the evaporation of the estrogen solution; they seem to be caused solely by the use of too vigorous a current of gas. A 2.0-ml. portion of reagent A (described subsequently) is added to the dried residues of the estrogens; then the tubes are covered in order to exclude moisture and heated on a steam bath for ten minutes. At the end of three minutes the tubes are shaken to assure complete dissolution of the estrogen residue. Finally the tubes are cooled in water, the contents transferred quantitatively to volumetric flasks, and made up to 10 ml. with reagent B. Care is necessary to ensure perfect mixing of the viscous reagents before transferring a sample to 1-cm. cells for light absorption measurements at 406 mμ, 456 mμ, and 506 mμ in a Beckman spectrophotometer. Triplicate standards are included with each batch of tubes. Standard ethanolic estrogen solutions were found to keep perfectly for at least one year when stored in a refrigerator in tightly stoppered flasks. Glass stoppers permit loss of alcohol by evaporation and consequently the solutions increase in strength. The estrogen content of the samples is calculated by Allen's formula (3):

$$CD_{456} = OD_{456} - \frac{OD_{406} + OD_{506}}{2}$$

where OD_n is the observed optical density at wave length n mμ, and CD_{456} is the optical density due to the estrogen at 456 mμ as defined by Allen. The following conditions are optimal for estriol: Reagent A consists of a mixture of four volumes of concentrated sulfuric acid with one volume of 90 per cent ethanol. Reagent B consists of two volumes of concentrated sulfuric acid and one volume of 90 per cent ethanol. These reagents deteriorate rapidly on exposure to air. They may be kept for several weeks in bottles with well-fitting glass stoppers. The magnitude of CD_{456} depends upon the exact composition of the reagents and the nature of the estrogen; the CD_{456} for estriol is about 0.13 for a solution containing the color from 1 μg. of the estrogen in 1 ml. The color is quite stable; the

optical density at 456 mμ decreases at the rate of 2 per cent per twenty-four hours.

Estrone and estradiol: The best reagents for estrone and estradiol contain larger proportions of ethanol and water, but the advantage gained is small. The CD_{456} values for estrone and estradiol are about 0.18 and 0.09 respectively, for solutions containing the color from 1 μg. of the estrogen in 1 ml.

RESULTS

The preservation of urine specimens: The estrogen content of several fresh urine specimens was determined and checked after these specimens had been stored for several months. Results are given in Table 1. The results of duplicate estrogen determinations made concurrently do not differ by more than 3 per cent. The increased variability found in stored samples probably is due to causes other than instability of the estrogens. It seems that urine specimens may be stored for two years at room temperature under a layer of butanol.

Cleavage of the estrogen conjugates of human pregnancy urine: Hydrolysis with aqueous mineral acid has been the universal means of cleaving the

TABLE 1. STABILITY OF ESTROGENS IN STORED URINE SPECIMENS

Sample	A	B	C	D	E	F
Time stored (months)	7	10	16	19	20	24
Initial estrogen content (mg. per 24 hrs.)	18.6	24.8	19.8	12.9	8.6	12.0
Final estrogen content (mg. per 24 hrs.)	19.3	26.6	19.7	9.2	7.8	13.2

estrogen conjugates in human pregnancy urine. If urine samples are hydrolyzed with acid for a given length of time and extracted at the end of that period, a maximum of recoverable estrogen is obtained at sixty to ninety minutes of hydrolysis. This maximum does not represent complete hydrolysis of the conjugated estrogens, as shown by the fact that urine samples which have been boiled for ninety minutes and extracted thoroughly with ether yield further small quantities of ether-soluble estrogen on continued hydrolysis. Yet if aliquots of the same samples are hydrolyzed for two to six hours before any ether extraction is made, the quantities of estrogen recovered are not increased; frequently the longer period of boiling results in a reduced yield of estrogens. These observations indicate that up to about sixty minutes of boiling, estrogen is liberated more rapidly from its conjugates than it is destroyed by the boiling acid; at sixty to ninety minutes the rate of liberation is approximately compensated by the rate of destruction; beyond ninety minutes the rate of destruction is dominant

and recoveries of estrogens decline. There are indications that with a few urine samples the optimal time of hydrolysis is considerably shorter. It is to be expected that the optimal conditions will depend largely upon the kinds and quantities of estrogen conjugates present.

The colorimetric assay of estrogen: Numerous modifications of Allen's reagent were tried, but none was markedly superior to the original for routine use. The relationship between the efficiency of the reagent and its chemical composition is puzzling: the 90 per cent ethanol of Allen's reagent may be replaced without change of efficiency by diethyl ether, dioxane, acetic acid, or acetic anhydride, but substitution of methanol for

TABLE 2. MEANS AND STANDARD DEVIATIONS OF URINARY ESTROGENS IN 9 CASES OF NORMAL PREGNANCY

Weeks of pregnancy	Estrogens (mg./24 hrs.)	Standard deviation
20	2.7	0.2
22	3.9	1.1
24	4.4	1.5
26	5.9	1.6
28	6.0	1.5
30	7.5	1.4
32	8.2	2.5
34	11.0	3.5
36	15.0	5.2
38	19.0	7.3
40	17.6	—

ethanol causes a 17 per cent reduction of the color intensity given by pure estriol. Similarly a change from diethyl ether to ethylene glycol diethyl ether causes a 70 per cent reduction of the color intensity; the efficiency of ethylene glycol dimethyl ether is between the two.

Some results of estrogen assays: The urinary estrogen values found in 9 cases of normal pregnancy are summarized in Table 2. These values are lower than those given by Venning (7). This discrepancy may be attributed at least partly to the fact that the urine samples had to be collected by patients without supervision. A study of the creatinine content of these "24-hour" urine specimens showed that many of them must have been incomplete (8). The creatinine corrections had to be based upon the average creatinine content of the urines furnished by each individual, authentic 24-hour specimens not being available. This necessarily led to over-all estrogen values which are too low.

SUMMARY

A modified method for the recovery of estrogens from human pregnancy urine is described, which is thought to combine a minimum of operations with an optimal recovery of estrogens. The urine specimens may be stored for two years at room temperature under a layer of *n*-butanol. It is shown that the optimum attainable does not constitute a quantitative recovery of the estrogens present in human pregnancy urine when aqueous mineral acid is used as the means of cleaving the conjugates.

The Allen modification of the Kober colorimetric assay was investigated and somewhat improved. This method is more convenient for routine use and subject to fewer errors (9, 10) than other modifications of the Kober method. The uncertainty inherent in this assay is estimated to involve at most 300 μg. per 24-hour urine specimen, depending upon the colored impurities present in the urine.

Acknowledgments

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ACID-HYDROLYZABLE CORTICOIDS OF SERUM*

R. KLEIN, M.D.,† C. PAPADATOS, M.D., J. FORTUNATO, M.S. AND CAMILLA BYERS, B.S.

The Department of Pediatrics, University of Pittsburgh Medical School and the Children's Hospital of Pittsburgh, Pittsburgh, Pennsylvania

SINCE the work of Nelson (1) there have been many studies concerning blood corticoids. These have raised questions concerning the exact significance of measurements of chloroform extractable blood corticoids, hereinafter called free corticoids. Were there conjugated as well as free blood corticoids? What was the significance of conjugated blood corticoids, if such existed? Were the free blood corticoids an insignificant part of the total blood corticoids and, if so, were their measurements of any value? Finally, the question arose, if there were conjugated blood corticoids, were they important as active hormones or could they be converted to active forms of hormone if they were inactive? The present study attempts to answer some of these questions.

METHODS AND SUBJECTS

The method used for the measurement of conjugated corticoids was essentially an adaptation of the method of Nelson and Samuels (1) for free 17,21-dihydroxy-20-ketosteroids in blood. Either plasma or serum was extracted according to their method and the free corticoids measured. The serum or plasma following the third extraction was then hydrolyzed with acid, and the material once more extracted according to the original method of Nelson and Samuels; and the acid hydrolyzable corticoids were then measured. Nearly all the measurements reported here were made on serum. The details for the method are as follows:

The residue from the Nelson-Samuels extraction of free corticoids is drained from the separatory funnel into a 250-ml. glass-stoppered centrifuge bottle. The separatory funnel is washed out with 10 ml. of concentrated hydrochloric acid (37 per cent) and this acid is drained into the centrifuge bottle. The bottle is then shaken in a mechanical shaker for fifteen minutes. The use of 25 ml. of 25 per cent hydrochloric acid instead of the 37 per cent hydrochloric acid has made no discernible difference in the results, but using the minimal amount of acid to lower the pH to 4.5, 2.0 or 1.0 gives lower results. After shaking, the glass stopper is removed from the centrifuge bottle with care and the pressure within the bottle relieved. Twenty-five ml. of chloroform, freshly distilled as in the original method of Nelson and Samuels, is added to the centrifuge bottle, which is then shaken once more in the shaking machine; the emulsion is broken by centrifugation.

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The lower level of chloroform is removed by aspiration with a 30-cc. syringe and a 4-inch 19-gauge needle. The chloroform is placed in an Erlenmeyer flask. This extraction process is repeated twice more and the chloroform extracts pooled in the Erlenmeyer flask. The pooled chloroform extract is evaporated under an air blast. The dried extract is redissolved in 5 cc. of chloroform and treated in exactly the same manner as the original Nelson-Samuels extracts. Florisil column chromatography was used throughout.

The subjects listed as normal for the study were laboratory workers, residents and medical students in apparent good health, as well as patients in the hospital for elective surgery or for the investigation of neurologic problems or mental retardation. There were also several children with mongolism who, although they were not included among the control subjects for the determinations of normal values during the day, were included among the control subjects for the cortisone tolerance tests, since their results did not differ in any way from those in other children of similar age.

The patients with rheumatic fever were all children who had been treated with 100 mg. of cortisone by mouth every eight hours for varying lengths of time. These patients were maintained on a 50-mg. sodium diet while receiving cortisone.

Blood specimens were also obtained from patients with acute infections, various liver diseases, hypothyroidism and renal disease. The relationship between the time the specimens were obtained and the clinical disease will be considered subsequently.

RESULTS

Methodologic. The colorimetric measurement of these extracts by the Nelson-Samuels (1) micromodification of the Porter-Silber reaction has been consistently satisfactory. The reproducibility of the results when duplicate specimens were analyzed on succeeding days was excellent. The variation of such duplicate specimens, or of duplicate specimens run simultaneously, was within 10 per cent. The addition of cortisone to serum or plasma did not affect the level of bound corticoids when the serum was extracted immediately or after an incubation period of twenty-four hours at 37° C. Further extraction of the original unhydrolyzed residues by shaking in the machine and centrifuging gave no additional free corticoid, even when the hydrolyzed residues then gave high conjugated corticoid values.

The absorption in the final color measurement of certain of these hydrolyzed specimens was too great for proper measurement. No investigation has been made as to whether hexane washing or any other means might be available to prevent this excess, as it is a rare occurrence. If the free corticoids are not first extracted and measured in the usual way, but the untreated serum or plasma is hydrolyzed and extracted, then either the readings are beyond the measurable range or the background colors are so high that the final result is a zero or negative value.

It has been observed that the free corticoid material in plasma survives well if the plasma is kept sterile and in the refrigerator (2). However, it has been noticed in this laboratory that the free corticoids deteriorate rapidly but irregularly if the plasma or serum is allowed to stand at room temperature in an ordinary test tube. This is also true of the conjugated

corticoids. When serum in a centrifuge bottle was immersed in a boiling water bath for from five to thirty minutes, reaching a maximal temperature of 90° C., there was an irregular increase in yield of the acid-hydrolyzable materials measured by the microadaptation of the Porter-Silber method. If the serum was allowed to remain in the water bath for longer periods there was a gradual disappearance of the conjugated corticoid. Some free corticoid was still measurable after all the conjugated corticoid had disappeared.

There was less than a 10 per cent loss of tetrahydro compound E when it was carried through the hydrolysis procedure described here. On the other hand, when standard compound F was carried through the same procedure, there was a loss of 50 per cent of the material.

Normal sporadic levels. Serum was obtained from 30 normal adults and 45 "normal" children at varying times during the day and with a varying relationship to meals. In general, the adult normal specimens were obtained just after breakfast. The average value for free corticoids in both groups was 13 gamma (μgm.) per 100 ml. of serum or plasma. This is the same value previously reported by the authors for another group of nor-

TABLE 1. AVERAGE FREE AND CONJUGATED SERUM CORTICOID LEVELS AND SEM BEFORE AND AT HOURLY INTERVALS AFTER THE INGESTION OF CORTISONE (60 MG./SQ.M.) BY ADULTS AND CHILDREN

(The differences between the results in children and adults are significant only at the 90 per cent level and only in those pairs marked with an asterisk.)

	Corticoids (γ/100 ml.)					
	Control	1 hr.	2 hrs.	3 hrs.	4 hrs.	8 hrs.
Adults						
Free	13±1.9	54± 1.7	50±13.2	37±5.4	21±1.5	9±2.6
Conj.	10±2.5	33± 1.7	41± 4.6*	33±6.5*	33±3.8	22±5.3
No. of samples	(13)	(16)	(4)	(7)	(3)	(9)
Children						
Free	14±2.8	65±10.4	48± 4.9	52±8.4	37±8.4	11±3.5
Conj.	6±1.8	31± 7.2	24± 5.7*	24±4.0*	32±5.6	20±6.1
No. of samples	(17)	(20)	(9)	(9)	(7)	(11)
Total						
Free	14	60	49	46	33	11
Conj.	8	32	29	31	32	21
No. of samples	(30)	(36)	(13)	(16)	(10)	(20)

* $P < 0.1$.

mal subjects (3). Similar values have been reported for children by Kelley and Ely (4) and for adults by Bliss *et al.* (5). The average value for serum conjugated corticoids in children was 4 gamma per 100 ml. (SEM[1] ± 1.1) and in adults, 8.5 gamma per 100 ml. (SEM ± 1.25). The difference between the two mean values is statistically significant ($P < .01$). The range in both adults and children was 0 to 27 gamma per 100 ml. of serum.

Cortisone tolerance tests in adults. Sixteen normal adults and 22 children who were called normal for the purposes of this study were given 60 mg. of cortisone per square meter of body surface area by mouth. The actual doses varied from 55 to 62 mg. per square meter because of the size of the standard cortisone pill. Blood specimens were drawn just before the adminis-

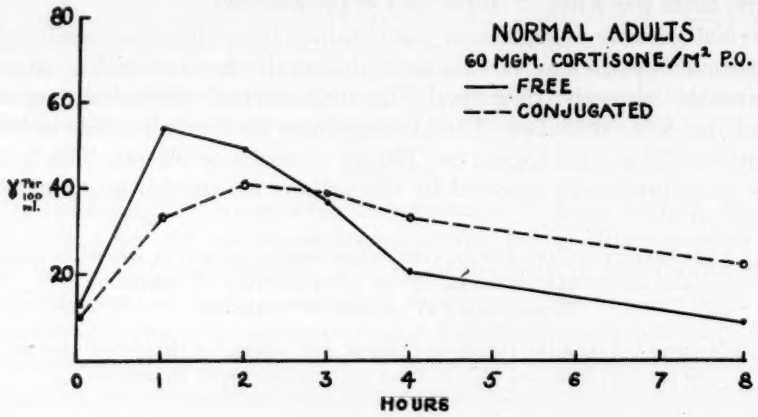

FIG. 1. Average free and conjugated serum corticoid levels before and after the ingestion of cortisone by 16 normal adults. The free corticoids are represented by the dots and solid lines, the conjugated corticoids by the circles and broken lines. The corticoid levels in gamma (μgm.)/100 ml. of serum are plotted against the time in hours.

tration of cortisone and at a varying number of hourly intervals thereafter. Not more than three or four blood specimens were drawn on any of the subjects (except the investigators) in any one study. Table 1 gives the results of these tests, showing the number of samples measured at each period of time, and the average level and SEM for both free and conjugated corticoids at each period of time. Figure 1 shows the average cortisone tolerance curves for both free and conjugated corticoids in adults. As may be seen, the range of variation was wide, partly due to varying rates of absorption. Nelson has shown that the peak level of free corticoids in the blood is usually reached about one hour after ingestion (6). However, this is not always so. A 6-year-old boy in whom corticoids were measured be-

[1] Standard error of the mean.

fore, and one, four and eight hours after cortisone ingestion may be considered as an example. Before cortisone the value for free serum corticoid was 2 gamma per 100 ml. and at this time the value for conjugated corticoids was 1 gamma per 100 ml. At one hour there had been no significant rise and the levels were 6 and 7 gamma per 100 ml., respectively. At four hours the levels were 44 and 28 gamma per 100 ml. and at eight hours they were 0 and 8 gamma per 100 ml. Apparently he had absorbed his cortisone slowly and his peak level came later than one hour.

Cortisone tolerance tests in normal children and infants. Similar dosages and times were used in cortisone tolerance tests in 22 "normal" children and infants. As may be seen from Table 1 the levels of free corticoid reached a higher average value at one hour than they did in adults receiv-

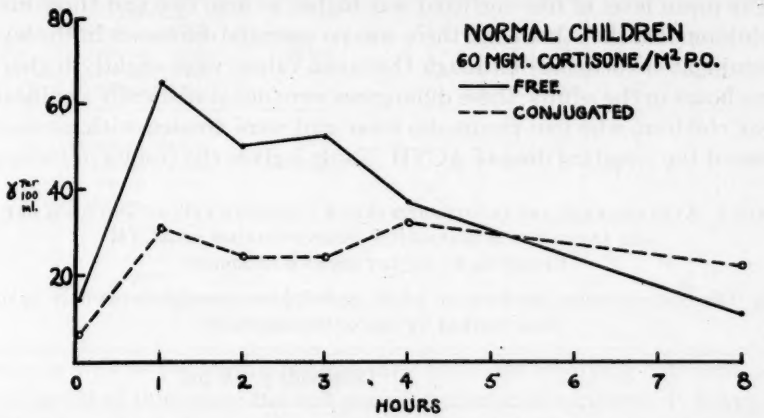

FIG. 2. Average free and conjugated serum corticoid levels before and after the ingestion of cortisone by 22 children. This graph is plotted in the same manner as Figure 1.

ing similar amounts of cortisone. The average level of conjugated corticoid at this point did not differ from that found in the adults. The levels of both free and conjugated corticoids at eight hours were also indistinguishable from those seen in adults. However, the levels of free corticoids were higher in children at the third and fourth hours than those found at the same time in adults, and the ratio of free to bound corticoids approached unity at a later time. The conjugated corticoid level in these children was lower at the second and third hours than at the corresponding time in adults. These differences were not statistically significant.

Children under the age of 2 years were considered separately from the children above this age, since infants have been shown to have lowered renal functions per unit of surface area and Marks (7) has recently shown that the excretion of at least the biologically active corticoids is directly

related to renal function, presumably to glomerular filtration. Only 9 infants were studied. There were no discernible differences between their levels and those found in older children. Figure 2 shows the average cortisone tolerance curves for both free and conjugated corticoids in all the children.

Adrenocorticotropic hormone (ACTH). ACTH in doses of 10 mg. per square meter was administered intravenously within three minutes to 18 adults and 17 children between the ages of 3 months and 40 years. Blood samples were drawn before, and one, two, or three hours after administration of ACTH.

Table 2 gives the results for both adults and children and Figure 3 shows curves for the mean results.

The mean level of free corticoid was higher at one, two and three hours in children. On the other hand there was no essential difference in the levels of conjugated corticoids although the mean values were slightly higher at three hours in the adults; these differences were not statistically significant.

Six children, who had rheumatic fever and were treated with cortisone, received the standard dose of ACTH. Table 3 gives the results in these pa-

TABLE 2. AVERAGE FREE AND CONJUGATED SERUM CORTICOID VALUES AND SEM BEFORE AND AFTER THE INTRAVENOUS ADMINISTRATION OF ACTH (10 MG./SQ.M.) TO ADULTS AND CHILDREN

(The differences between the levels in adults and children are significant only in those pairs marked by one or two asterisks)

	Corticoids γ/100 ml.			
	Control	1 hr.	2 hrs.	3 hrs.
Adults				
Free	12±2.3	30±1.8*	28±4.5	30±4.1
Conj.	8±1.8**	11±1.9	14±1.9	20±2.8
No. of samples	(18)	(15)	(12)	(9)
Children				
Free	13±1.7	39±3.3*	35±6.0	35±5.1
Conj.	4±1.1**	10±1.6	17±4.5	16±1.9
No. of samples	(17)	(16)	(9)	(7)
Total				
Free	12	35	31	32
Conj.	6	11	15	18
No. of samples	(35)	(31)	(21)	(16)

* $P < 0.1$.
** $P < 0.01$.

TABLE 3. FREE AND CONJUGATED SERUM CORTICOID VALUES IN 6 CHILDREN WITH RHEUMATIC FEVER, BEFORE AND AFTER THE INTRAVENOUS ADMINISTRATION OF ACTH (10 MG./SQ.M.)

Children with rheumatic fever	Treatment	Time since last dose	Corticoids γ/100 ml.							
			Control		1 hr.		2 hrs.		3 hrs.	
			Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.
WA	Cortisone 300 mg., 50 dys.	8 hrs.	28	42	23	47			44	35
MA	Cortisone 300 mg., 27 dys.	8 hrs.	51	20	43	37			22	30
DO	Cortisone 300 mg., 7 dys.	8 hrs.	42	65	32	21			38	33
DA	Cortisone 300 mg., 48 dys. 200 mg., 2 dys. 150 mg., 2 dys. 100 mg., 1 dy.	12 hrs.	0	8	5	3	3	6		
PL	Cortisone 300 mg., 44 dys. 200 mg., 2 dys. 150 mg., 2 dys. 100 mg., 1 dy.	12 hrs.	7	0	9	5	14	9		
AD	Cortisone 300 mg., 18 dys. ACTH 100 mg., 10 dys.	40 dys. 41 dys.	0	0	16	5	27	13		

tients, as well as the length of time they had been receiving cortisone and the interval of time since the last previous intake of cortisone. It is apparent that these high dosages of cortisone inhibited the free corticoid response to ACTH but did not inhibit the conjugation of corticoid.

Renal failure. A blood specimen was obtained from a 10-year-old boy with acute cortical necrosis, after ten days of anuria. He had, however, undergone a vivodialysis three days before. This specimen contained no free corticoids but did contain 115 gamma of conjugated corticoids per 100 ml. Blood was also obtained from 2 boys with chronic nephritis and azotemia, one month and one week respectively, before death. The patients, 6 and 12 years old, were voiding about 500 ml. daily when the specimens were obtained but had blood nonprotein nitrogen levels of 120 and 180 mg. per 100 ml. The analyses showed 10 gamma of free and 28 gamma of conjugated corticoids per 100 ml. in the first patient, and corresponding values of 6 and 125 gamma in the second.

Diabetic coma. The average free corticoid level found in 7 patients with diabetic coma on the day of admission was 71 gamma per 100 ml., and the mean conjugated corticoid level was 36 gamma. Twenty-four hours later

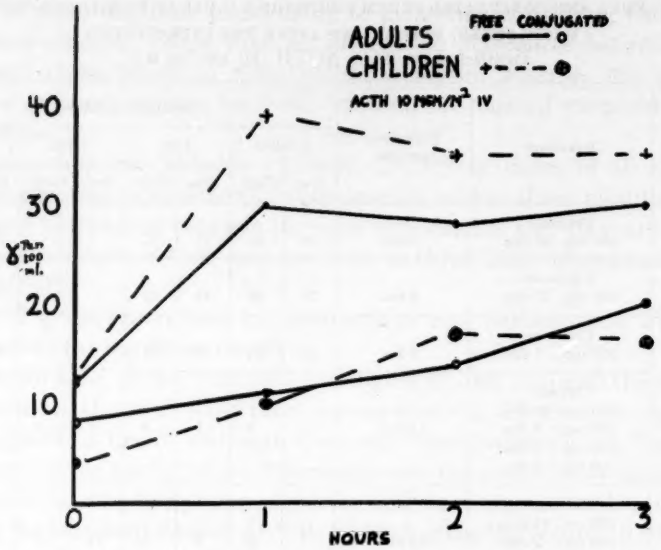

FIG. 3. Average serum free and conjugated corticoid levels before and after the administration of ACTH. The values for the 18 adults are represented by solid lines, those for 17 children by broken lines. For the adults, the free corticoid values are represented by the dots and the conjugated corticoid values by the circles. For the children the corresponding data are represented by crosses and by circles, respectively.

the levels were 24 and 21 gamma per 100 ml., respectively.

Meningitis. On the day of admission 11 patients with acute pyogenic meningitis had an average free corticoid level of 69 gamma and an average conjugated corticoid level of 25 gamma per 100 ml. of serum. Twenty-four hours later 4 of these children had levels that averaged 13 and 24 gamma per 100 ml., respectively. One other patient, a 7-year-old boy, was admitted to the hospital because of meningococcemia and meningococcic meningitis. His blood pressure was normal on admission. An intravenous infusion of physiologic saline and glucose was started, and penicillin and sulfadiazine were given by this route. Twenty minutes thereafter he became unresponsive and his blood pressure was unobtainable. His pulse was rapid and thready, his color was poor, and he was considered to be in shock. Blood drawn at this time, just before the administration of cortisone intravenously, contained 16 gamma of free corticoid and 67 gamma of conjugated corticoid per 100 ml. The boy recovered uneventfully. Another patient was dead on arrival at the hospital. She was shown to have meningococcic meningitis with massive bilateral adrenal hemorrhage at postmortem examination. Blood obtained from the heart on arrival con-

TABLE 4. SERUM CORTICOID VALUES BEFORE AND AFTER ANESTHESIA AND SURGICAL STRESS IN 7 PATIENTS

(The times refer to the interval from the beginning of anesthesia)

Pt.	Age (yrs.)	Corticoids/100 ml.									
		Pre-anesth.		½ hr.		1 hr.		2 hrs.		4 hrs.	
		Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.
MI	36	8	6	22	9	34	2	45	18		
FR	8	9	5	26	6	25	17			61	40
LU	3	27	9			33	12				
SA	6	27	6	42	6						
AL	12	9	7	6	5						
GI	14	10	7	20	2	36	7				
M-U	13	2	3	18	4						
HA	15	9	6	28	8						

tained 53 gamma of free and 34 gamma of conjugated corticoid per 100 ml.

Surgical trauma. The average pre-anesthesia levels for free and conjugated corticoids in 19 patients were 11 and 6 gamma per 100 ml. These rose to averages of 51 and 21 gamma four hours after the beginning of anesthesia. There were 7 adults and 12 children in this group and the only difference noted was that the adults had a slightly higher control conjugated corticoid level than did the children. In addition to the control measurements, 3 children and 1 adult had blood samples drawn at approximately one-half and one hour after the beginning of anesthesia. Four children had single post-anesthesia measurements at one-half hour. The results in these 7 individuals are tabulated in the Table 4. Table 5 shows the average for the whole group. It is apparent that the rise in free corticoids preceded the rise in conjugated corticoids.

Liver disease. In patients with advanced cirrhosis there is practically no

TABLE 5. AVERAGE SERUM CORTICOID LEVELS BEFORE AND AFTER ANESTHESIA AND SURGICAL STRESS

(The times refer to the interval from the beginning of anesthesia)

	Pre-anesth.		½ hr.		1 hr.		2 hrs.		4 hrs.	
	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.	Free	Conj.
No. of patients	(19)		(7)		(4)		(1)		(13)	
%	11	6	23	6	32	10	45	18	51	21

conjugation of administered corticoid. These patients and patients with hepatitis will be the subject of a separate communication, as their corticoid levels differ in other ways as well (8).

Rheumatic fever. The serum corticoid response to orally administered cortisone did not seem to differ in 24 children with rheumatic fever from that seen in "healthy" children.

Hypothyroidism. Two 4-year-old girls with hypothyroidism, who had a remnant of functioning thyroid tissue, responded normally to ACTH. Conjugation of the endogenous corticoid was excellent in one and low-normal in the other.

DISCUSSION

The rates of recovery obtained by the method used in this work are analogous to the results obtained by using a similar method of hydrolysis of urine. The pitfalls are obvious, but the method has the advantages of being easy to carry out on large numbers of specimens and having good reproducibility. For the purposes of this study, the consistency of the response curves was more important than the exact magnitude of conjugated corticoid in any individual.

The rise in the level of conjugated serum corticoids follows that of free serum corticoids when the patient undergoes surgical stress or receives ACTH or cortisone. The elevation of conjugated corticoids persists longer than that of free corticoids after cortisone administration or after the stress of acute meningitis or diabetic coma. It was obviously not possible to begin the studies soon enough in diabetic coma and meningitis to ascertain if the rises in the corticoid levels took place in the same order, nor were the studies after ACTH administration and after operation carried out long enough to see if the conjugated corticoid level remained elevated after that of free corticoid fell. At the moment there seems to be no reason to doubt that the relationships of free and conjugated corticoids are similar in both respects in all these different situations. In acute and chronic liver disease there is inhibition of the conjugation of exogenous and endogenous corticoid, although in other diseases (diabetes, meningitis, rheumatic fever and chronic nephritis) this does not occur.

In renal failure there is an extreme elevation of the level of conjugated corticoids in the presence of low or normal values for free corticoids.

From these observations, it seems likely that the material measured by the methods reported here consists of corticoids that have been conjugated by the liver, are probably inactive, and are candidates for excretion by the kidney. It would be expected that these materials are largely tetrahydro compound E, since this compound forms the bulk of the recoverable conjugated urinary corticoid (9). As such, the importance of determining

conjugated corticoids in evaluating reaction to stress at the time blood is obtained is probably negligible. Although it is only one instance, the example of the patient with meningococcemia who was in a state of shock in the hospital would tend to support this view. It may well be that the limiting factor in the rate of corticoid conjugation is not the process of conjugation itself, but the rate of necessary metabolism of the corticoid before it can be conjugated.

Children have sporadic levels of serum conjugated corticoids that in our small series were statistically significantly lower than similar adult levels, although the levels of free corticoids in both adults and children were the same. Since, at least beyond the age of 2 years, renal function in children is proportionate to that of adults, the rate of excretion of corticoids should not account for a difference in the disappearance rate of the corticoids. Hence the conjugation of corticoids in children seems to be less rapid than in adults. Since the level of free corticoids tends to be higher in children given ACTH or cortisone than in adults, even when the conjugated corticoid level has finally reached the adult value, it is likely that the rate of degradation of serum corticoid in other ways is also lower in children than in adults.

The differences in normal conjugated corticoid levels between children and adults and the differences after cortisone ingestion and ACTH administration are in the same direction. However, except for the differences in normal sporadic conjugated corticoid values, these changes are either not statistically significant or are significant only at the 90 per cent level of confidence. This aspect of the work, therefore, requires confirmation. In addition, any attempt to use institutionalized children as controls must be regarded with a certain amount of suspicion.

SUMMARY

A method which apparently measures acid-hydrolyzable conjugated serum corticoids has been presented. Assays have been made in normal individuals before and after the administration of ACTH or cortisone, and similarly in individuals under the acute stress of infection, surgical procedures, or diabetic coma. In addition, such measurements were made in children with rheumatic fever receiving cortisone therapy, and in patients with such disorders as liver dysfunction, or renal failure. These studies suggest that the conjugated corticoids are formed in the liver and excreted in the urine. They are presumably inactive. The rise in the blood level of conjugated corticoids after stress or cortisone ingestion follows that of the free corticoids. Except in renal failure, when values for conjugated corticoids rise out of proportion to the previous values for free corticoids, the level of serum conjugated corticoids reflects the previous level of free corticoids.

Acknowledgments

The authors wish to express their thanks to Dr. Alfred Bongiovanni, without whose suggestions and advice this work would not have been carried out. Above all, they wish to thank the residents and interns of the Children's Hospital of Pittsburgh, who gave so freely of their time, energy and blood. In addition the authors gratefully acknowledge their debt to Dr. Richard Cyert for his help with the statistical analyses, to Dr. Lawrence Greenman for obtaining blood on patients in diabetic coma and to Dr. S. Richard Bauersfeld for permission to study patients with rheumatic fever who were under his care.

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THYROID-ADRENOCORTICAL METABOLIC INTERRELATIONS*†

IRA S. GOLDENBERG, M.D., LEO LUTWAK, PH.D., PAUL J. ROSENBAUM, M.S. AND MARK A. HAYES, M.D., PH.D.

The Samuel C. Harvey Metabolic Unit, Department of Surgery, Yale University School of Medicine, New Haven, Connecticut

THE exact metabolic interrelationships between the thyroid and the adrenal cortex have been subject to many varying interpretations. Experimental evidence from animal investigations suggests that adrenocortical products are antagonistic in some way to thyroid hormone, as demonstrated by a decrease in nitrogen excretion following the administration of adrenocortical hormone to dogs previously rendered hypermetabolic by the ingestion of thyroxin (1). The present study was designed to evaluate several metabolic activities in 2 patients at varying levels of thyroid and adrenocortical function in a controlled environment.

MATERIALS AND METHODS

The first subject (E.McC.) was a hypothyroid female with presumably normal adrenocortical function, who was studied to determine the metabolic effects of maximal adrenocortical stimulation (by exogenous ACTH) during periods of hypothyroidism and hyperthyroidism. The second subject (M.H.) was a euthyroid male with hypoadrenocortical reactivity, as shown by lack of response to the ACTH-eosinophil test (Thorn). He was studied to ascertain if any metabolic changes were induced by administration of aqueous adrenocortical extract during periods of depressed and elevated thyroid function.

Daily each subject consumed all of a basic diet (1,600 calories), which included 10 grams of nitrogen, 2 grams of sodium and 2,000 milliliters of tap water per 1.73 square meters of body surface (2). Appropriate caloric and fluid deductions were made from the daily oral intake when replaced by parenteral fluids necessary for the conduct of the investigation.

Twenty-four-hour urine specimens were collected and stored under refrigeration, each collection starting at 8 A.M. daily. Total nitrogen (3) excretion was determined as an index of general metabolic activity, and total inorganic phosphorus (3) excretion, which

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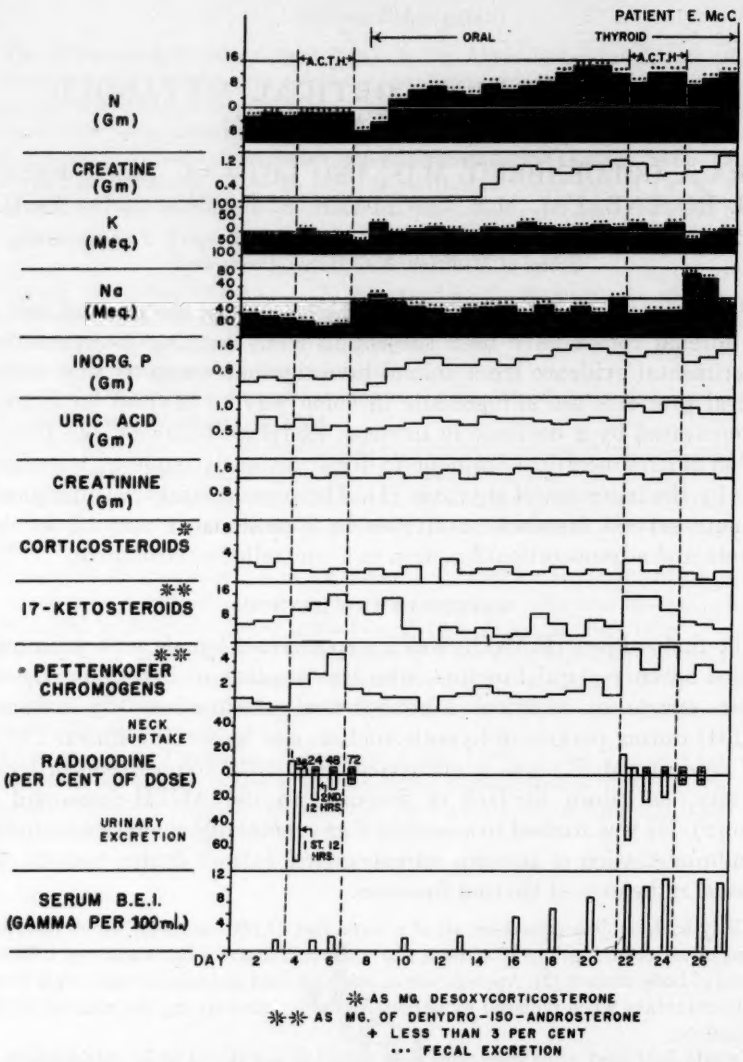

FIG. 1. *Patient E.McC.* Metabolic data charted according to the method of Reifenstein *et al.* (22). Numbers over bars in radioiodine chart refer to the number of hours after administration of I^{131} that counts were taken. See text for discussion.

parallels that of nitrogen, was measured as a check on the nitrogen values. Urinary creatine was determined as an indicator of the level of thyroid activity (4), and uric acid (5) to denote the level of nucleoprotein metabolism. Thyroid activity additionally was determined by the degree of concentration in the neck region of an orally administered tracer amount of radioiodine (I^{131}) and by the urinary excretion of this isotope. Radio-

activity was measured for 4-minute periods, utilizing a highly efficient, bismuth-coated cathode tube and making the necessary corrections for background activity and extrathyroidal iodine. Data on concentration and excretion of I^{131} are expressed as a percentage of the total amount of radioiodine administered. Thyroidal I^{131} concentration was determined at one, three, six and twenty-four hours after administration and every twenty-four hours thereafter for three days. Urinary excretion was determined for two 12-hour periods following the ingestion of the isotope and then for 24-hour periods until there was no remaining radioactivity detectable in the urine. When I^{131} was administered more than once within a short period of time, the newer comparison standard solution included the amount previously given (from the original dilution) to correct for residual glandular radioactivity. The butanol-extractable iodine (BEI) (6) content of the postabsorptive serum aided in evaluating thyroid activity.

The urinary excretions of 17-ketosteroids (7, 8), corticosteroids (9, 10) and Pettenkofer chromogens (7, 11, 12) were determined as indicators of adrenocortical activity. The corticosteroids are expressed as milligrams of desoxycorticosterone, whereas the 17-ketosteroids and the Pettenkofer chromogens are expressed as milligrams of dehydroisoandrosterone. The uric acid/creatinine ratio was also calculated as another index of the level of adrenocortical activity, albeit a crude one (13).

Feces, collected for 3-day and 4-day periods, were frozen at −20 degrees F. and were later analyzed for nitrogen, sodium and potassium (14). Each collection period was demarcated by the ingestion of a carmine-red capsule which stained the feces.

RESULTS

Patient E. McC. (Figs. 1 and 2) was a 54-year-old woman with a nonfunctioning malignant tumor of the thyroid, who had had a total thyroidectomy and left radical neck dissection performed one year prior to admission. A hypothyroid state was induced before starting the present study by discontinuing for a month the maintenance thyroid medication of 0.2 Gm. daily. During a period of equilibration on the basic diet a slightly positive nitrogen balance existed, creatine excretion was stable (determined at 0.07 Gm. daily), urinary steroid metabolites were relatively stable, the uric acid/creatinine ratio averaged 0.5, and the serum BEI level was 2 gamma per 100 ml.

Maximal adrenocortical stimulation was attempted by the administration of 20 units of adrenocorticotropic hormone (ACTH) in a liter of 5 per cent glucose in water intravenously over an 8-hour period daily for three consecutive days (15). A tracer amount of radioiodine (90 microcuries) was given coincidentally with the start of the first infusion. The 24-hour concentration of I^{131} by remnants of the thyroid gland remaining in the neck was less than 3 per cent, and the urinary excretion during the first twenty-four hours after administration was approximately 90 per cent.

Excretion of 17-ketosteroids and Pettenkofer chromogens increased significantly, but corticosteroid excretion remained essentially constant under ACTH stimulation, as did the uric acid/creatinine ratio. Marked

sodium retention and a moderate potassium diuresis were noted. Nitrogen excretion did not change significantly during the ACTH administration. No important alterations occurred in other urinary constituents and it is particularly important to note that the hyperadrenocortical state had no effect on the urinary excretion of creatine during the period of hypometabolism.

Desiccated thyroid medication was then started orally at a daily dose

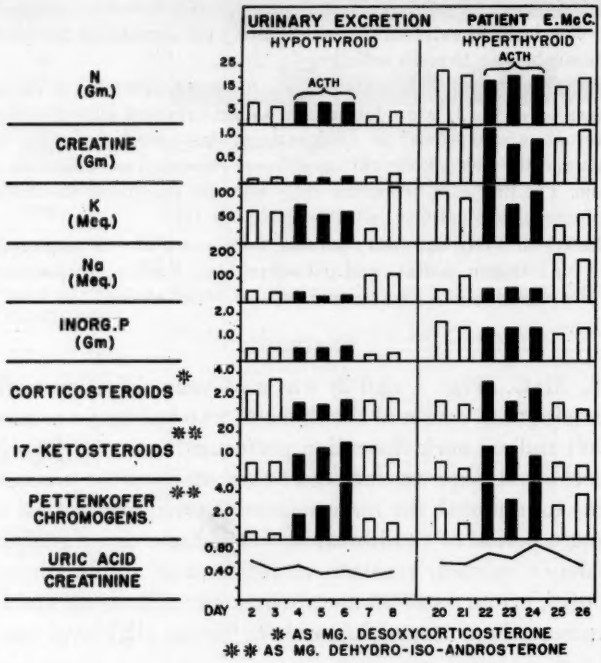

FIG. 2. *Patient E. McC.* All data charted show absolute urinary excretions, with the exception of the uric acid/creatinine ratio, which is expressed as a reduced fraction. Each bar represents a 24-hour collection period. The days during which ACTH was administered are depicted by the solid black bars. See text for discussion.

level of 0.1 Gm. and was gradually increased over a period of thirteen days to 0.8 Gm. daily. Repeated serum BEI determinations revealed a slow increase to hyperthyroid levels (11 gamma per 100 ml.) by the fifteenth day of administration. During this period no clinical manifestations of hyperthyroidism were noted, although the patient did complain of some "nervousness." A marked creatinuria, with values of over 1.0 Gm. daily, was observed by the eighth day of thyroid administration. Urinary inorganic phosphorus increased slowly to between two and three

times the hypothyroid level and a negative nitrogen balance developed slowly, continuing throughout the period of thyroid medication.

When a state of hypermetabolism had been achieved, ACTH was again administered as previously and a tracer dose of I^{131} (84 microcuries) was given. A slight but not significant increase in the uptake in the neck region was noted when compared to a previous test, but the 24-hour urinary excretion did not change. Nitrogen excretion (Fig. 1) decreased during the ACTH infusions, whereas all urinary steroid metabolites increased. The uric acid/creatinine ratio was elevated to 0.8 and there was a moderate sodium retention and potassium diuresis. The increase in adrenocortical activity under the same stimulation was slightly greater when a hyperthyroid state existed than when hypometabolism was present. Creatine excretion decreased during the second series of infusions of ACTH. Fecal excretions of sodium, potassium and nitrogen remained constant during this study and apparently were unaffected by the various readjustments occurring in this patient.

Patient M.H. (Figs. 3 and 4) was a 55-year-old man, with pulmonary tuberculosis, first diagnosed in 1947. During the succeeding years several operative procedures were performed and periods of chemotherapy instituted. During one hospitalization the patient was found to be unresponsive on two occasions to the ACTH-eosinophil (Thorn) test for adrenocortical function. No signs or symptoms of adrenocortical insufficiency were present, however, and no hormone therapy had been given.

The patient was started on his calculated diet. A dose of 48 microcuries of radioiodine administered orally at this time revealed a euthyroid response, with 24 per cent concentration by the gland after twenty-four hours. The serum BEI level was 4.9 gamma per 100 ml. There was a slightly negative nitrogen balance, and creatine excretion approximated 0.1 Gm. daily. Potassium balance was negative and sodium balance was positive. The urinary excretion of steroid metabolites was stable except for daily variation in the Pettenkofer chromogens. The uric acid/creatinine ratio averaged 0.3. It should be noted that despite a lack of response to the ACTH-eosinophil (Thorn) test, adrenocortical steroid metabolite levels of definite significance were present in the urine during this investigation.

Exogenous thyroid was then administered, starting with a dose of 0.3 Gm. daily and rapidly increasing to a total dose of 1.06 Gm. daily, in an attempt to create a hypermetabolic state. Serial determinations of the serum BEI concentration revealed a rapid increase, so that by the ninth day after starting thyroid medication the level was 11.8 gamma per 100 ml. A creatinuria of over 0.4 Gm. daily was present and nitrogen excretion increased 50 per cent, so that a marked negative nitrogen balance was created. Only 8 per cent of a dose of 51 microcuries of I^{131} administered

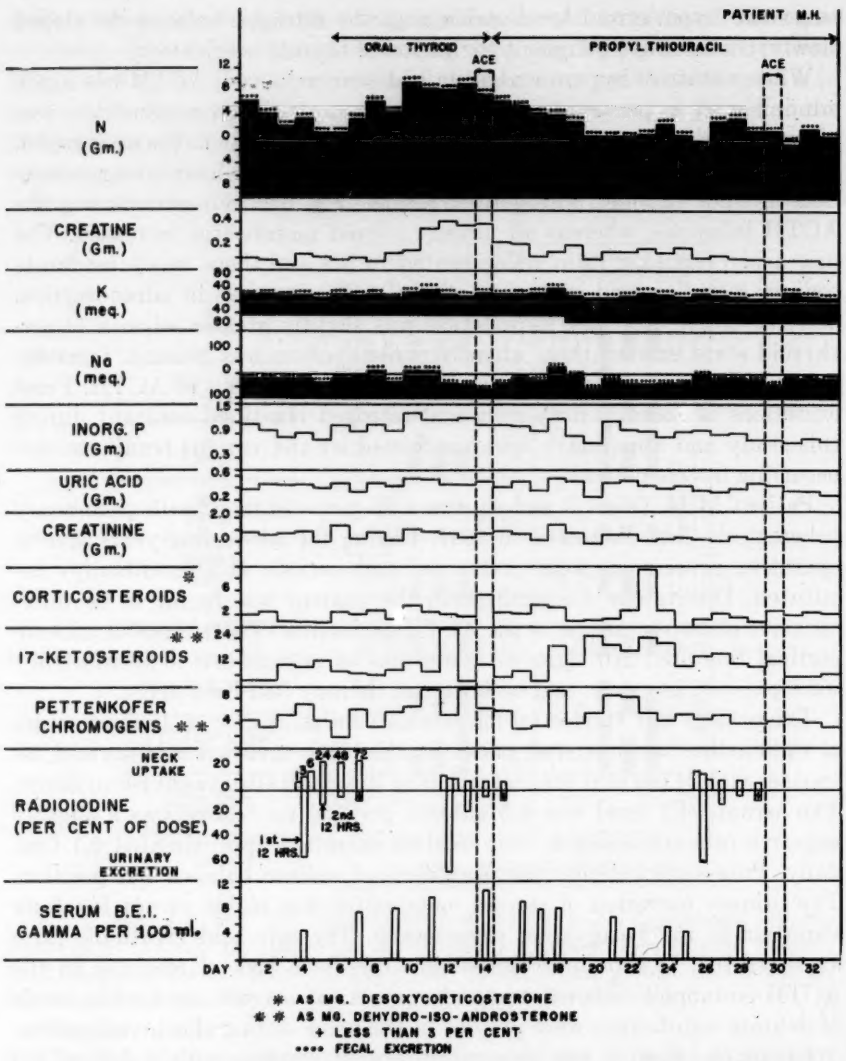

FIG. 3. *Patient M. H.* Data are charted as in Figure 1. See text for discussion.

orally at this time was concentrated after twenty-four hours by the thyroid gland, indicating a marked suppression of glandular activity by the exogenous hormone which had been given during the preceding period.

During the state of hypermetabolism (day 14) an infusion of 300 milliliters of aqueous adrenocortical extract (ACE) in a liter of 5 per cent glu-

cose in water was given over an 8-hour period (8 A.M.–4 P.M.). In order to delineate more sharply the transient metabolic effects of ACE, urine collections were made for 12-hour instead of 24-hour periods during the day before the infusion and the day of the infusion. In this way an 8 A.M.–8 P.M. control period was established to compare with the ACE period, and

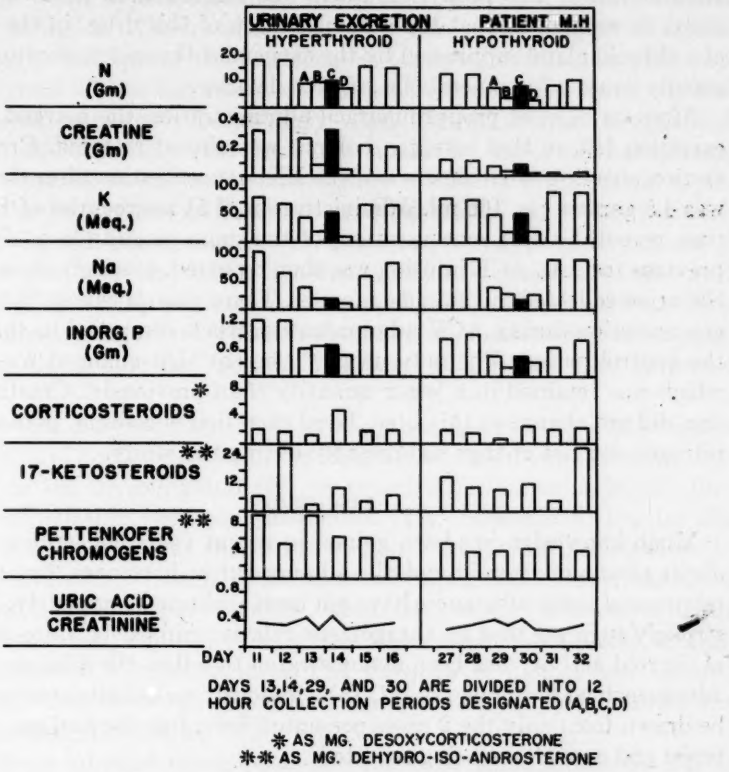

FIG. 4. *Patient M. H.* Data are charted as in Figure 2. Period "C" represents the time during which the ACE was administered. Period "A" serves as the control for period "C," and period "B" serves as the control for period "D." All adrenocortical steroid determinations are for 24-hour periods. See text for discussion.

in like manner a second control period (8 P.M.–8 A.M.) was demarcated to compare with the period following the infusion.

Nitrogen excretion (Fig. 4) was not significantly altered during the period of ACE administration, but immediately following the infusion it was sharply reduced when compared to the control period. A slight potassium diuresis and a moderate sodium retention were evident during the

infusion, but the urinary steroid metabolites were essentially unchanged except for an increased corticosteroid excretion during the ACE administration. It is important to note that a sharp fall in urinary creatine followed the ACE infusion.

Thyroid medication was discontinued the day following the ACE administration and propylthiouracil was started in a dose of 800 mg. daily. It was hoped that the administration of this drug, in the presence of a thyroid gland suppressed by the exogenous thyroid medication, would rapidly cause a hypometabolic state to develop.

After six days of propylthiouracil administration the marked nitrogen excretion fell, so that nitrogen balance was almost restored. Creatine excretion also decreased slowly and the BEI concentration after eleven days was 4.5 gamma per 100 ml. Administration of 51 microcuries of I^{131} at this time revealed a low concentration, of the same magnitude as during the previous test. An ACE infusion was then repeated, precisely as before, and the urine collected in the same manner. There was no change in the nitrogen excretion during ACE administration when compared to that during the control period. The only urinary element that changed was sodium, which was retained in a lesser quantity than previously. Creatine excretion did not change at this time. Fecal excretion of sodium, potassium and nitrogen did not change significantly during the study.

DISCUSSION

Much knowledge has been gained in recent years concerning the metabolic effects of thyroid and the adrenocortical hormones, but the interrelations of these substances have not been evaluated completely. Evidence strongly suggests that an antagonistic relationship exists, since depression of thyroid activity has been demonstrated to follow the administration of adrenocortical hormone (16, 17, 18). Obviously no definite conclusions can be drawn from only the 2 cases presented here, but the findings are of interest and as such are being reported.

The present investigation supports a viewpoint of antagonism. During the period of hypermetabolism and marked negative nitrogen balance in Patient E.McC., maximal adrenocortical stimulation by ACTH resulted in a markedly increased urinary excretion of all adrenocortical steroid metabolites measured, sodium retention, and a sharp increase in the uric acid/creatinine ratio. During this adrenocortical response, depression of thyroid effects occurred, as manifested by a greatly decreased creatinuria. It is most important to note that, in addition, a marked decrease in urinary nitrogen occurred. On the other hand, at the time this patient was hypometabolic, adrenocortical response also followed ACTH administration, but there was no indication at this time that thyroid activity was affected,

since the urinary excretion of creatine and of nitrogen did not change.

It is of interest to note the differential response of the adrenal cortex to stimulation by ACTH in this patient during each phase of thyroid activity. When hypometabolism was present, urinary 17-ketosteroids and Pettenkofer chromogens increased, whereas corticosteroids remained essentially constant. When hypermetabolism was present, however, urinary corticosteroids as well as 17-ketosteroids and Pettenkofer chromogens increased.

Thus it would appear that endogenous adrenocortical oxysteroids suppress thyroid effects and cause a decrease in urinary nitrogen; but this phenomenon apparently occurs only when thyroid activity is at a high level. A previous study (19) from this laboratory revealed that adrenocortical response at any moment probably is directly proportional to the level of thyroid activity at that time. In other words, when body metabolism is depressed, adrenocortical effects are not noted, despite evidence of adrenocortical activity. When hypermetabolism is present, on the other hand, adrenocortical hormones lead to profound and apparently protective metabolic alterations. These findings lend themselves well to an interpretation founded on the "permissive action" of adrenocortical hormones (20).

It has been suggested by some investigators that the mode of action of adrenocortical hormones in counteracting thyroid hormone effects is either directly on the thyroid gland (21), or represents suppression of pituitary thyroid-stimulating hormone production (17). Strong evidence for the concept that at least one site of interaction is at the cellular level is the decrease in urinary creatine which occurred during ACTH administration in Patient E.McC. Urinary creatine, which is a sensitive indicator of tissue metabolism levels, diminished at a time when exogenous thyroid medication remained at a constant level. The viewpoint is further corroborated by the fact that the serum BEI concentration did not change during the adrenocortical stimulation.

Exogenous adrenocortical hormone causes the same metabolic alterations noted following stimulation of endogenous production. This is illustrated by the results in the study of Patient M.H., in whom aqueous adrenocortical extract administered during the hypothyroid phase did not alter thyroid metabolic effects; creatine and nitrogen excretion did not decrease. When hyperthyroidism and marked negative nitrogen balance were present, however, a sharp fall in urinary creatine was noted during the 12-hour period following the ACE infusion, and nitrogen excretion was significantly lowered during this same period.

A decrease in urinary nitrogen was observed immediately after ACTH infusions given to Patient E.McC. during the hypermetabolic phase. This decrease was undoubtedly the result of pre-existent adrenocortical

hypertrophy following maximal stimulation by ACTH. Adrenocortical steroid metabolites in the urine and the uric acid/creatinine ratio continued to be elevated at this time, indicating a persistence of adrenocortical effects. The same results obtained, however, following the ACTH infusions given during the hypometabolic phase, except that corticosteroids were not increased during or after the infusions. Why a decrease in urinary nitrogen occurred here following ACTH administration is not clear. It is possible that other adrenocortical factors which were not measured in this study may affect nitrogen excretion when maximal adrenocortical activity is present and when thyroid activity is low.

SUMMARY

Adrenocortical hormones apparently are antagonistic to thyroid metabolic effects in man, the site of hormonal interaction probably being the cellular metabolic level. This interaction may be related closely to the so-called permissive action of adrenocortical steroids.

Acknowledgments

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SIMULTANEOUS MEASUREMENT OF THE IODIDE-CONCENTRATING AND PROTEIN-BINDING CAPACITIES OF THE NORMAL AND HYPERFUNCTIONING HUMAN THYROID GLAND*

SIDNEY H. INGBAR, M.D.

The Department of Biophysics, Army Medical Service Graduate School, Walter Reed Army Medical Center, Washington 12, D. C., and the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and Harvard Medical School, Boston, Massachusetts

IT IS generally agreed that the remarkable ability of the thyroid gland to accumulate iodine depends upon two successive, though perhaps related actions of the gland. First, inorganic iodide from the plasma is highly concentrated within the gland and second, thyroidal iodide is oxidized and bound to an organic moiety (1–5). When either of these functions is abolished, the thyroid gland fails to accumulate or to retain iodine (6–8). Since both appear to be rate-limiting factors in the production of thyroid hormone, methods which measure over-all iodine accumulation must reflect their combined activity. It would, therefore, appear useful to measure each of these functions individually, and to assess their importance in the initiation of abnormal, or the maintenance of normal thyroidal function.

Methods have been devised for the measurement *in vivo* of the ability of the gland to concentrate inorganic iodide. Such methods depend on the complete inhibition of protein-binding of iodine, using pharmacologic agents or large doses of inorganic iodine (9, 10). Measurement of the rate of protein-binding of concentrated iodide has not been possible, however. This measurement must rest upon a knowledge of the quantity of concentrated iodide, a quantity which heretofore could not be determined unless protein-binding were completely inhibited.

The method herein presented makes possible, in addition to the measurement of the over-all rate of iodine accumulation, the simultaneous estimation of the ability of the gland to concentrate inorganic iodide and of the rate of conversion of iodide to organically-bound iodine. Normal

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values for these functions have been determined in 28 euthyroid subjects, and their relative importance in the production of increased thyroid function has been assessed in 20 patients with untreated Graves' disease. The data reveal that the increased thyroidal function of Graves' disease is usually the result of an increased thyroidal mass, an increased ability of a unit mass of thyroid tissue to concentrate inorganic iodide, and an increased capacity of the gland to convert inorganic iodide to hormonal iodine.

METHOD

The method is based upon the following assumptions regarding the metabolism of iodine during the period of study. Their validity is discussed subsequently.

1. The magnitude of the thyroidal iodide space remains constant.
2. Exchange of inorganic iodide between the thyroid and the plasma is very rapid. The specific radioactivity of iodide in the plasma is, at any moment, equal to that in the thyroid gland.
3. A constant percentage of thyroidal inorganic iodide is converted to hormonal iodine per unit time.
4. Release of newly-formed radioactive hormone is negligible.
5. Radioiodide disappears from the plasma at an exponential rate.

Let t = time (hours) after administration of I^{131}.
I_B = concentration of inorganic I^{131} in the plasma at time, t (% dose/liter).
$I_{B_0} = I_B$, when t = 0 (extrapolated value).
TIS = Thyroidal iodide space (liters). (Thyroid/plasma iodide concentration ratio × thyroidal mass; total thyroidal iodide/plasma iodide concentration).
I_C = amount of I^{131} in TIS at time, t (% dose).
I_H = amount of protein-bound I^{131} in thyroid at time t (% dose).
I_T = total amount of I^{131} in thyroid at time, t (% dose).
I_U = ultimate or asymptotic ($t = \infty$) uptake of I^{131} (% dose).
K = rate of binding of thyroidal iodide to protein (%/hour).
k = rate constant of disappearance of I^{131} from the plasma (proportion/hour).
T_T = thyroidal rate of transfer of plasma iodide to hormonal iodine (liters/hour).
IDS = iodide distribution space (liters). (Administered dose I^{131}/I_{B_0}.)

At any time, t, the concentration of radioiodide in the plasma,

$$I_B = I_{B_0}e^{-kt} \qquad 1$$

and the total amount of radioiodine within the thyroid,

$$I_C = \text{TIS } I_{B_0}e^{-kt}. \qquad 2$$

At any time, the rate of increase of hormonal I^{131} within the thyroid gland,

$$dI_H/dt = KI_C \qquad 3$$

$$= K \text{ TIS } I_{B_0}e^{-kt}. \qquad 4$$

The amount of hormonal I^{131} within the gland at any time

$$I_H = -\frac{K \text{ TIS } I_{B_0}e^{-kt}}{k} + C.$$

Since when $t=0$, $I_H=0$,

$$C = \frac{K\ TIS\ I_{B_0}}{k}$$

and

$$I_H = \frac{K\ TIS\ I_{B_0}(1 - e^{-kt})}{k}. \qquad 5$$

The total amount of I^{131} within the gland is the sum of organic and inorganic radioiodine.

$$I_T = I_C + I_H. \qquad 6$$

From equations 2 and 5,

$$I_T = TIS\ I_{B_0}e^{-kt} + K\ TIS\ I_{B_0}/k - K\ TIS\ I_{B_0}e^{-kt}/k. \qquad 7$$

Therefore, the slope of the iodine uptake curve,

$$dI_T/dt = -k\ TIS\ I_{B_0}e^{-kt} + K\ TIS\ I_{B_0}e^{-kt}$$
$$= TIS\ I_{B_0}e^{-kt}(K - k). \qquad 8$$

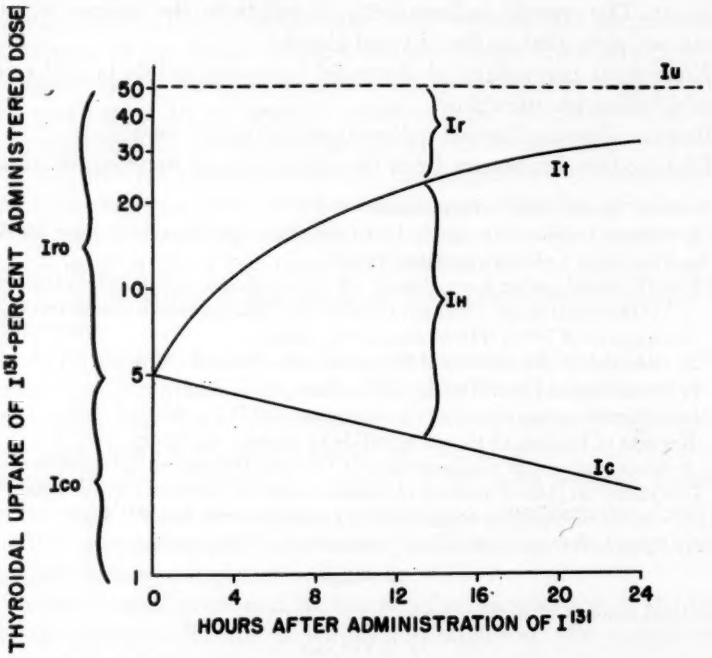

FIG. 1. Schema of thyroidal collection of I^{131} in a patient in whom the rate of loss of radioiodide from the thyroid is less than its rate of incorporation into hormone. I_U = asymptotic ($t=\infty$) value of radioiodine uptake. I_T = thyroidal radioiodine content at any time, t. I_{C_0} = "instantaneous" uptake of trapped radioiodide. I_{R_0} = amount of radioiodine remaining to be taken up by the thyroid when $t=0$. I_H = thyroidal content of protein-bound I^{131}. Both I_{C_0} and I_{R_0} decrease at the same exponential rate, k, as does the plasma radioiodide concentration (not shown).

If the accumulation of a single dose of I^{131} is observed for an infinitely long period ($t = \infty$),

$$I_B = 0; \qquad I_C = 0; \qquad (1 - e^{-kt}) = 1.$$

At this time, all radioiodide has disappeared from both the plasma and the thyroid gland, and the gland's radioiodine content is entirely protein-bound. Therefore, when $t = \infty$

$$I_T = I_U = I_H$$

and

$$I_U = \frac{K\ TIS\ I_{B0}}{k}. \qquad 9$$

Equation 9 states that the maximal amount of I^{131} collected by the thyroid gland is directly proportional to the initial plasma concentration of radioiodide, to the thyroidal iodide content relative to the plasma concentration, and to the ability of the gland to convert inorganic to organically-bound iodine. It is inversely proportional to the rate at which radioiodide disappears from the plasma.

If mixing of injected I^{131} and specific activity equilibrium between plasma and thyroidal iodide are instantaneous, there will occur, following the injection of I^{131}, an instantaneous uptake of radioiodide into the thyroidal iodide space. This instantaneously "trapped" iodide will be called "I_{C_0}." From this time onward, accumulation of radioiodine in the thyroid proceeds as illustrated in Figure 1.

$$I_{C0} = TIS\ I_{B_0}.$$

From equation 2,

$$I_C = I_{C_0}e^{-kt}. \qquad 10$$

If I_R = the amount of I^{131} (% dose) which the gland has yet to take up, and I_{R_0} = the value of I_R when $t = 0$

$$I_U = I_{C_0} + I_{R_0} = I_T + I_R \qquad 11$$

$$I_R = I_U - I_T. \qquad 12$$

Therefore, from equations 9 and 7,

$$I_R = \frac{K\ TIS\ I_{B_0}}{k} - \left(\frac{K\ TIS\ I_{B_0}}{k} - \frac{K\ TIS\ I_{B_0}e^{-kt}}{k} + TIS\ I_{B_0}e^{-kt}\right)$$

$$= \frac{K\ TIS\ I_{B_0}e^{-kt}}{k} - TIS\ I_{B_0}e^{-kt} \qquad 13$$

$$dI_R/dt = -K\ TIS\ I_{B0}e^{-kt} + k\ TIS\ I_{B_0}e^{-kt}$$

$$= TIS\ I_{B_0}e^{-kt}(k - K) \qquad 14$$

$$\frac{dI_R/dt}{I_R} = \frac{TIS\ I_{B_0}e^{-kt}(k - K)}{\frac{K\ TIS\ I_{B0}e^{-kt}}{k} - TIS\ I_{B_0}e^{-kt}}$$

$$= \frac{k - K}{\frac{K}{k} - 1}$$

$$= -k. \qquad 15$$

I_R therefore decreases by the same exponential rate, k, as does I_B, and

$$I_R = I_{R_0}e^{-kt}. \qquad 16$$

However, from equations 6 and 11,

$$\begin{aligned} I_H &= I_T - I_C \\ &= I_U - I_R - I_C \\ &= I_U - I_{R_0}e^{-kt} - I_{C_0}e^{-kt}. \end{aligned}$$

The rate of change of glandular hormonal I^{131},

$$\begin{aligned} dI_H/dt &= kI_{R_0}e^{-kt} + kI_{C_0}e^{-kt} \\ &= ke^{-kt}(I_{R_0} + I_{C_0}) \\ &= ke^{-kt}I_U. \end{aligned} \qquad 17$$

From equations 3, 10, and 17, the organic-binding rate,

$$\begin{aligned} K &= \frac{dI_H/dt}{I_C} \\ &= ke^{-kt}I_U/I_{C_0}e^{-kt} \\ &= kI_U/I_{C_0} \qquad 18 \\ &= kI_U/\text{TIS}\ I_{B_0}. \qquad 19 \end{aligned}$$

Equation 19 is identical to equation 9.

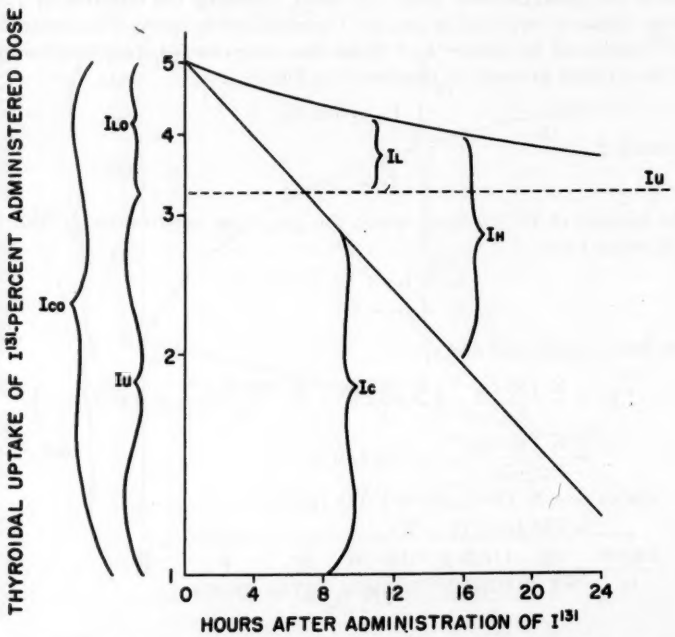

FIG. 2. Schema of thyroidal collection of I^{131} in a patient in whom the rate of loss of radioiodide from the thyroid exceeds its rate of incorporation into hormone. I_U = asymptotic ($t = \infty$) value of radioiodine uptake. I_T = thyroidal radioiodine content at any time, t. I_{C_0} = "instantaneous" uptake of trapped radioiodide. I_{L_0} = amount of radioiodine remaining to be lost by the thyroid when $t = 0$. I_H = thyroidal content of protein-bound I^{131}. Both I_{C_0} and I_{L_0} decrease at the same exponential rate, k, as does the plasma radioiodide concentration (not shown).

It is now possible to apply the foregoing considerations to the calculation of these functions in man. A tracer dose of I^{131} is given intravenously. Serial plasma samples are taken, and from these k and I_{B_0} are determined. Repeated determinations of the percentage uptake of I^{131} by the thyroid are made and from these, a curve of uptake is constructed. Two points in time are selected, t_1 and t_2. From equations 1, 12 and 16,

$$\frac{I_{B_1}}{I_{B_2}} = \frac{I_{R_1}}{I_{R_2}} = \frac{I_U - I_{T_1}}{I_U - I_{T_2}}.$$

Since I_{B_1}, I_{B_2}, I_{T_1} and I_{T_2} are known, I_U and hence I_{R_1} and I_{R_2} can be calculated. From equations 1 and 16,

$$\frac{I_{R_0}}{I_{R_1}} = \frac{I_{B_0}}{I_{B_1}}.$$

I_{R_0} is calculated. However, by equation 11

$$I_{C_0} = I_U - I_R$$

TIS can be calculated since

$$TIS = I_{C_0}/I_{B_0}.$$

The binding rate K is then calculated by equation 18,

$$K = kI_U/I_{C_0}.$$

It will be seen from equation 8, that the slope of the radioactive iodine uptake curve will be negative when k exceeds K, *i.e.*, when the rate of loss of radioiodide from the plasma (and the thyroidal iodide space) exceeds the rate of conversion to hormonal iodine of I^{131} within the thyroidal iodide space. Curves of this type, most commonly seen when K is reduced by pharmacologic agents, can also be analyzed according to the foregoing considerations (Fig. 2).

If I_{L_0} = total amount of I^{131} (% dose) remaining to be lost from the thyroid gland when $t = 0$,

$$I_L = I_T - I_U. \qquad 20$$

In a manner similar to that for I_R (equations 13–16), I_{L_0} can be shown to decrease by the same rate constant, k, as I_B and I_C; therefore

$$I_L = I_{L_0}e^{-kt}. \qquad 21$$

However,

$$\begin{aligned} I_H &= I_T - I_C \\ &= I_U + I_L - I_C \\ &= I_U + I_{L_0}e^{-kt} - I_{C_0}e^{-kt}. \end{aligned}$$

Therefore,

$$\begin{aligned} dI_H/dt &= kI_{C_0}e^{-kt} - kI_{L_0}e^{-kt} \\ &= ke^{-kt}(I_{C_0} - I_{L_0}) \\ &= ke^{-kt}I_U. \end{aligned}$$

As in equation 18,

$$K = kI_U/I_{C_0}.$$

From equations 1, 20 and 21, for any two points in time, t_1 and t_2,

$$\frac{I_{B_1}}{I_{B_2}} = \frac{I_{L_1}}{I_L} = \frac{I_{T_1} - I_U}{I_{T_2} - I_U}$$

and

$$\frac{I_{L_0}}{I_{L_1}} = \frac{I_{B_0}}{I_{B_1}}.$$

I_{L_0} is determined, and

$$I_{C_0} = I_{L_0} + I_U.$$

From this point, the analysis proceeds as for uptake curves with an ascending slope.

According to equations 16 and 21, the thyroidal content of I^{131} should approach its limiting value at the same exponential rate as does the concentration of radioiodide in the plasma. This may be ascertained in the individual patient by plotting against time values of I_R and I_L for each of the observed points on the thyroidal accumulation curve. The slope of the resulting curve and of that depicting the plasma's concentration of radioiodide should be equal. In all of the patients in the present study, excellent agreement was noted in the slopes of the two curves.

ANALYSIS OF THE ASSUMPTIONS AND THE METHODS

The present method constitutes an attempt to measure discrete functions of the thyroid gland, heretofore unmeasurable in man. Before proceeding to a discussion of results, it would be well to examine the validity of the postulates upon which the method is based.

In living animals, including man, as well as in surviving thyroid slices, when organic binding of iodine is inhibited, the thyroid gland is capable of markedly concentrating inorganic iodide (1–3, 9, 11). This "trapped" inorganic iodide has been characterized by direct measurement of thyroid/plasma concentration ratios for both stable and radioactive iodide, by chromatographic and potentiometric characteristics, and by the ability of certain anions, including thiocyanate and perchlorate, to bring about its discharge from the gland (2, 6, 10, 11). More recently, thyroidal concentrating of inorganic iodide has been shown to be an active process, susceptible to abolition by anoxia, cooling, and a variety of metabolic inhibitors (12, 13).

Whether concentration differentials for inorganic iodide occur in the unblocked thryoid gland may legitimately be questioned. The weight of evidence suggests that they do. Agents, such as thiocyanate, which interfere with the ability of the blocked thyroid gland to concentrate inorganic iodide, produce goiter when given to animals not receiving blocking agents (6, 14). The goiters thus produced may be entirely prevented by the concomitant administration of large amounts of iodide, in contradistinction to goiters resulting from the administration of agents which inhibit organic binding (14). In addition, there is patently no impairment of blood flow to the highly vascular goiters thus produced. It may therefore be concluded that in the unblocked gland, thiocyanate goiter results from the abolition of thyroid/plasma concentration differentials for inorganic iodide, with subsequent limitation of substrate for the organic-binding process. A

similar mode of action must be invoked to explain the efficacy in the treatment of thyrotoxicosis of agents which inhibit iodide trapping (15).

Biopsies of thyroidal tissue in normal patients have demonstrated inorganic iodide in concentrations markedly in excess of the normal concentration of iodide in the plasma (16). Furthermore, direct measurements in living animals and surviving thyroid slices have demonstrated that when organic binding is allowed to proceed, concentration gradients for inorganic I^{127} and I^{131} are established (17–20). That such gradients do not result from analytical error or artifacts of fractionation is suggested by progressively decreasing concentrations of inorganic I^{131}, despite increasing concentrations of organically-bound isotope (17–19). Following an adequate equilibration period, constant gradients are obtained, despite decreasing concentrations of radioiodide in plasma or medium (17–19). In the rat, changes in concentration gradients induced by hypophysectomy or by the administration of TSH are qualitatively similar in both blocked and unblocked glands (18). Furthermore, concentration differentials in unblocked thyroid slices are decreased or abolished by thiocyanate, perchlorate, anoxia, cold, and by metabolic inhibitors which abolish gradients in the blocked thyroid gland (19). The radioiodine thus concentrated has been shown to be chromatographically indistinguishable from inorganic iodide (19). The same evidence which verifies the existence of an iodide trap in the blocked thyroid therefore demonstrates a similar mechanism in the unblocked gland.

In living animals, including man, the rate of loss of radioiodide from the blocked thyroid gland is approximately the same as from the plasma (2, 3, 9). Following an adequate period of equilibration, constant thyroid/plasma concentration ratios for iodide have been found in rats, mice, and chick embryos (18, 20, 21). These observations suggest that radioiodide in the blocked gland is in rapid exchange with that in the plasma.

Constant concentration ratios for iodide have also been observed in unblocked thyroid glands of rats (18). Lein has demonstrated that in the unblocked thyroid gland of rabbits, there occurs a rapid initial accumulation of inorganic radioiodine, followed by a progressive decrease in glandular radioiodide concentration, similar to the decrease in the radioiodide concentration of plasma (17). The present as well as previous studies have demonstrated that, in man, the thyroidal content of radioiodine approaches a maximum at the same exponential rate at which the plasma's concentration of radioiodide approaches a minimum (22). If it is granted that inorganic iodide taken into the gland from the plasma acts as the substrate for organic iodinations, it must follow that the radioactivity in the inorganic fraction of thyroidal iodine must also decrease by the same rate constant. The foregoing observations suggest that in the unblocked thy-

roid gland, as in the blocked, thyroidal and plasma radioiodide are indeed in equilibrium.

In the present method, it is assumed that organic binding of thyroidal iodide proceeds at a constant, finite, proportional rate. It has been suggested by others that organic binding may be instantaneous. This view, although appealing in its simplicity, is not consonant with many observations. If binding were instantaneous, it would be impossible to demonstrate inorganic iodide within the gland, since there is no reason to believe that a portion of thyroidal iodide is not available for organic binding. The existence in the unblocked gland of demonstrable concentration gradients for inorganic iodide would therefore appear to make instantaneous binding exceedingly unlikely.

Determination of the percentage of total thyroidal radioiodine which is organically bound at a given time interval after the administration of I^{131} provides an indication of the rapidity of the organic-binding reactions. In hypophysectomized rats, this percentage has been found to be less than normal (23). The conclusion that organic binding is slowed after hypophysectomy is in accord with the results of other investigators (24, 25). It is difficult to believe that binding is infinitely rapid in the normal rat and slowed after hypophysectomy. It would follow from that hypothesis that the normal rate of secretion of TSH would be precisely that which is required to keep binding instantaneous. Such an interpretation would also require that increases above normal in the rate of manufacture of thyroid hormone, induced by TSH, would necessarily be mediated in no part by increased rapidity of organic binding.

The author can find no evidence to justify *a priori* conclusions about the absolute magnitude of the organic-binding rate. For the reasons cited, however, it does not appear to be infinitely rapid.

Small doses of carrier iodide increase the concentration of inorganic iodide in the plasma without diminishing the thyroidal iodide space (9). The resulting increase in total thyroidal iodide does not alter the rate or amount of accumulation of concomitantly administered I^{131} (26). The assumption appears justified, therefore, that organic binding proceeds at a constant proportional rate which is, within limits, independent of the absolute concentration of iodide within the gland.

If organic binding is not instantaneous and concentration gradients for inorganic iodide exist in the unblocked gland, it should be possible to demonstrate a discharge of thyroidal radioiodide in man, following the administration of agents which abolish the iodide trap. Although, as noted previously, this has repeatedly been demonstrated in animals and thyroid slices by chemical fractionation, it was recognized that in man, such demonstration might prove more difficult.

As pointed out by Childs *et al.*, in the unblocked gland organically-bound I^{131} soon preponderates over inorganic radioiodine, and demonstration of this relatively small inorganic fraction might be obscured during rather gross external counting procedures (9). Simple calculations make possible an estimate of the amount of potentially dischargeable radioiodide present in the gland at varying intervals following the administration of I^{131}. Using average normal values for TIS, plasma concentration of radioiodide, and radioiodide disappearance rate, it may be calculated that 4.6, 4.2 and 3.8 per cent of the administered dose would be present in the euthyroid gland at thirty, sixty and ninety minutes respectively, following the administration of I^{131}. Similar calculations in hyperthyroid patients give an average of 15.5, 9.4 and 5.7 per cent at the same time intervals. It thus appears that discharge could be best demonstrated in hyperthyroid patients, in whom TIS is large. However, the discharging agent must be administered relatively soon after the I^{131}, since the specific radioactivity of the plasma iodide, and presumably of thyroidal iodide, decreases so rapidly in thyrotoxic patients.

Furthermore, amounts of thiocyanate which may safely be administered to patients, may not produce sufficiently high concentrations in the plasma to bring about complete and rapid discharge of thyroidal iodide. If distributed in 20 liters, 1.5 Gm. of NaSCN would produce maximum concentrations of approximately 1×10^{-3} M. In surviving thyroid slices concentrations of 10^{-2} M have been found necessary to discharge thyroidal iodide completely (13). In man, the rapid intravenous administration of 3.0 Gm. of NaSCN has failed to abolish completely salivary concentrating of I^{131} (27).

If in man, as in the unblocked thyroid slice, discharge of inorganic iodide is not instantaneous (19), continued organic binding of progressively decreasing quantities of radioiodide would obscure the expected decline in total thyroidal radioactivity. This difficulty would be particularly marked when organic binding is rapid. Thus the thyrotoxic patient is in some respects the most suitable, and in other respects the least suitable, for this endeavor. Finally the administration of thiocyanate, when performed with appropriate caution, must be carried out over a finite period during which continued trapping and binding of iodide could occur.

For these reasons it was considered that evidence concerning the presence of dischargeable thyroidal iodide would be of a lower order of validity when obtained by external counting techniques than when obtained by chemical fractionation and direct analysis of thyroidal radioiodide. In addition, positive demonstration of dischargeable iodide would be more significant than failure to demonstrate this moiety.

Despite these difficulties, it has been possible to demonstrate discharge-

able radioactivity in 5 of 6 hyperthyroid patients given 1.5 Gm. of NaSCN intravenously over a four-minute period beginning one half to one hour after the administration of I^{131} (Fig. 3). Furthermore, administration of this agent has on two occasions been followed by an increase in the concentration of I^{131} in the plasma, suggesting a return of inorganic radioiodide to the plasma from extravascular sites in which it had been concentrated. Recently Kirkland has also demonstrated the discharge of a portion of thy-

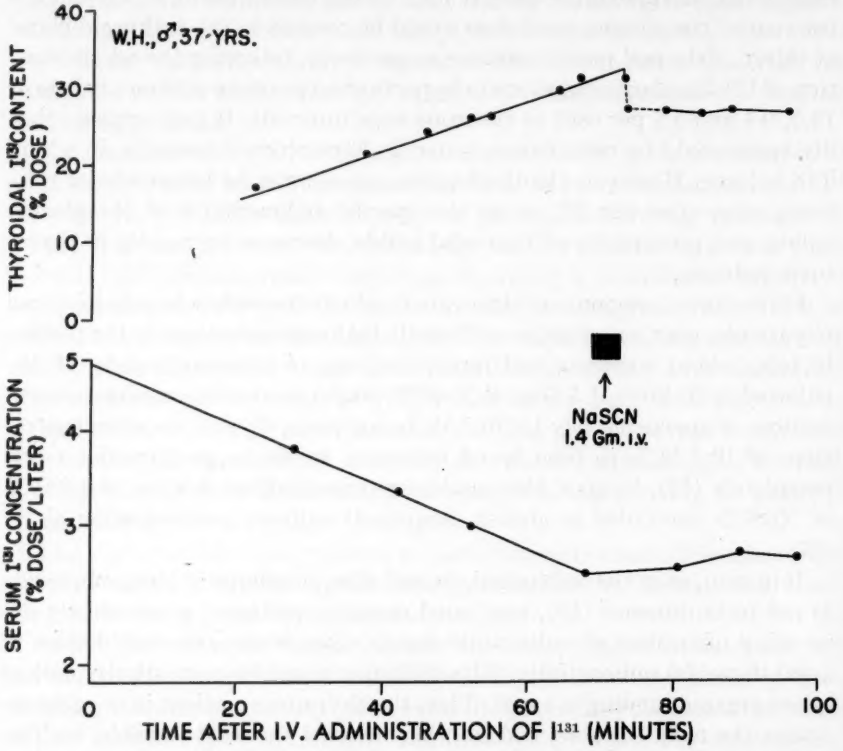

FIG. 3. Discharge of thyroidal radioiodine following the intravenous administration of 1.5 Gm. of NaSCN. Note the increase in the concentration of I^{131} in the plasma coincident with the decline in thyroidal radioactivity.

roidal radioiodine following the administration of thiocyanate to normal and thyrotoxic subjects (28).

In the derivation of the present method the concept of a rapid or "instantaneous" uptake of radioiodide into the thyroidal iodide space (I_{C_0}) has been introduced. It is obvious that instantaneous equilibration of radioiodide between thyroid and plasma does not occur. Wollman has demon-

strated that a finite period is required for concentration ratios to assume an equilibrium value (5, 20). This period represents the time required for the thyroidal circulation to deliver to the gland a quantity of radioiodide sufficient for the establishment of equilibrium ratios.

Similarly it has been assumed that intravenously administered I^{131} mixes instantaneously within its entire distribution compartment. This too is obviously not the case. I_{B_0} represents the concentration which plasma radioiodide would assume were equilibration of injected iodide instantaneous. It is a hypothetical value, obtained by extrapolation to zero-time of post-equilibration values of the plasma radioiodide concentration curves. Nevertheless, it has proved to be a useful means of closely approximating the volume of distribution of radioiodide (29), and similar methods have been used to determine dilution of many other labelling agents (30). In the same sense, I_{C_0} is a mathematically or graphically extrapolated value of the radioiodide content of the thyroidal iodide space, were equilibration of inorganic I^{131} between plasma and thyroid instantaneous.

Until injected I^{131} is uniformly mixed throughout its total space, the concentration of radioiodide in the plasma will exceed that predicted by extrapolation to zero-time of the post-equilibration curve. Thus, "disproportionate sampling" resulting from delay in the extrathyroidal mixing of I^{131} will tend to increase calculated values for I_{C_0} (31). On the other hand, failure of thyroid/plasma concentration gradients to reach equilibrium values instantaneously will tend to decrease calculated values for I_{C_0}. Thus errors in the determination of I_{C_0}, introduced by delays in mixing of radioiodide in the thyroidal and extrathyroidal iodide spaces are, to an unknown extent, mutually compensating.

The extent of the error introduced by mixing-phenomena into the calculation of I_{C_0} should be dependent on the magnitude of the total iodine-accumulative function of the gland. In individual patients, wide variations in the iodine-accumulative function, induced by propylthiouracil, were unaccompanied by material alterations in I_{C_0} (32). It may therefore be assumed that mixing-phenomena introduce relatively small errors in the calculation of the functions herein described.

I_{C_0}, although hypothetical, is not without its counterpart in reality. Following the administration of I^{131}, there occurs a rapid initial uptake of radioiodine into the thyroid. In less than one minute measurable quantities of radioactivity have penetrated the gland. This rapid uptake, noted by previous observers (17, 33), assumes a greater magnitude in hyperthyroid than in euthyroid patients, presumably as a result of both a more rapid circulation to the gland and an increased capacity to concentrate iodide. Organic-binding processes apparently do not contribute materially, since its magnitude is unaltered by the administration of propylthiouracil. It

has repeatedly been possible, however, to bring about virtual abolition of this early rapid uptake by the prior administration of thiocyanate. The phenomenon would appear, therefore, to be a reflection of iodide trapping and not of extrathyroidal radioactivity (Fig. 4).

In euthyroid individuals, significant quantities of radioactive hormone do not appear in the blood for several hours following the administration of a tracer dose of I^{131} (34). Since the test herein described is completed within two and one-half hours, it is unlikely that the results are materially altered by release of newly formed radioactive hormone. In patients with

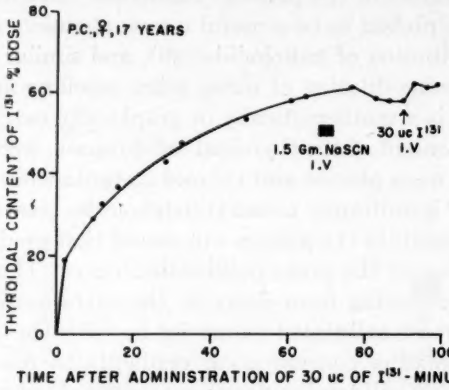

FIG. 4. The "instantaneous" uptake of trapped radioiodide in a patient with untreated Graves' disease. Within the first two minutes after the intravenous injection of I^{131}, 19 per cent of the administered dose was present in the region of the thyroid. Following the administration of 1.5 Gm. of sodium thiocyanate, a second dose of I^{131} produced only a slight increase in the radioactivity in the thyroidal region.

hyperthyroidism, radioactive hormone appears in the blood more rapidly than normal (34). However, the more rapid disappearance of I^{131} from the plasma of hyperthyroid patients, and its more rapid accumulation in the thyroid gland, make possible the collection, over a much shorter time period, of sufficient data to allow construction of accurate thyroidal and plasma radioactivity curves.

Procedure

A tracer dose of 50 microcuries of carrier-free inorganic I^{131} in isotonic saline is withdrawn from a sterile ampoule into a 5-cc. calibrated syringe. This volume of solution is delivered intravenously and an equal amount is delivered from the same syringe into a 50-cc. volumetric flask, which is subsequently used as a counting standard. The patient remains recumbent throughout the entire procedure. A shielded Geiger-Mueller tube (bismuth-sputtered-cathode) is placed 20 cm. from the skin surface of the neck for the determination of thyroidal radioactivity. The radioactivity detected 20 cm. from the

surface of the thigh is subtracted from that detected over the neck in order to "correct" for extrathyroidal radioactivity. Field plots have demonstrated that, with the shielding used in this laboratory, direct radiation from the superior mediastinal structures is not measured. Each group of measurements consists of two five-minute observations over the neck and one five-minute observation over the thigh. In patients with markedly increased function, two three-minute periods are used in determining thyroidal radioactivity. The frequency with which groups of measurements are made depends upon the level of thyroidal activity. In euthyroid subjects, six groups of observations over thyroid and thigh can conveniently be made within a two and a half to a three-hour period. In patients with hyperthyroidism, a similar number of observations can be made in from one to one and a half hours. Blood samples are withdrawn from the antecubital vein of the arm contralateral to that into which the I^{131} has been injected. Early studies revealed that in euthyroid patients, an exponential slope of disappearance of radioiodide from the serum was entered at forty to sixty minutes following the administration of I^{131}. Consequently, in subsequent studies, the first blood sample was not withdrawn before this time. In hyperthyroid individuals, the exponential phase is apparently entered earlier than in patients with normal thyroidal function. This may represent a more rapid mixing of iodide in such patients, or may indicate that the component of the declining slope related to thyroidal uptake of radioiodide preponderates over that component related to mixing. Four blood samples have proved to be adequate to define accurately the exponential curve of plasma radioactivity. The radioactivity of 0.5 cc. of samples of serum was determined with a thin (1.4–1.8 mg./cm.^{2}) mica-windowed Geiger-Mueller tube, and was compared with that of an appropriately diluted sample of the original counting standard. At least 10,000 net counts were recorded for each sample. A correction for self-absorption was made in the observed radioactivity of each sample.

RESULTS AND DISCUSSION

One hundred and seventy-five tests have been carried out in 150 individuals, including normal and hyperthyroid subjects, and patients with diverse abnormalities of thyroidal function.

Tables 1 and 2 summarize the results obtained in 28 patients without thyroid disease and in 20 patients with untreated Graves' disease. All patients who had received previous therapy for hyperthyroidism, and those with toxic adenoma were excluded.

Prediction of the 24-hour uptake (Table 3)

By means of equation 7, it is possible to predict the thyroidal uptake of radioiodine at any hour following the administration of the tracer. In 29 euthyroid subjects, the mean deviation of the predicted 24-hour uptake from the observed uptake was 0.5 per cent of the administered dose. In hyperthyroid patients, the predicted values exceeded observed values by a mean deviation of 10.8 per cent of the administered dose.

In none of the hyperthyroid patients did the predicted values fail to exceed the observed value. In these subjects, the radioactive iodine uptake had reached and passed its peak by the end of twenty-four hours. The data lend further support to the contention that the 24-hour radioiodine uptake

TABLE 1. EUTHYROIDISM

Patient	Age	Sex	TIS (liters)	T/P* ratio	K (% TIS/hr.)	T_T† (cc./min.)	C_T (cc./min.)	IDS (liters)	ETIDS (liters)	IDS/Kg. Body Wt. (cc.)	ITT (hrs.)
B.A.	28	F	1.3	44	112.7	25.0	19.3	19.2	17.9	338	12.8
B.Am.	23	F	1.4	54	103.6	23.5	17.5	20.0	18.6	366	14.2
M.A.	22	F	1.1	45	77.9	14.7	9.6	21.4	20.3	406	24.2
E.B.	32	F	1.0	40	124.0	21.3	14.8	14.7	13.7	267	11.5
I.B.	45	F	1.1	44	60.0	11.1	6.7	18.5	17.4	271	27.8
J.B.	24	F	0.8	40	71.5	9.5	5.7	16.6	15.8	317	29.2
T.B.	23	F	0.9	37	109.0	16.9	13.4	16.8	15.9	308	16.7
G.C.	31	F	0.8	41	78.6	10.6	8.3	19.7	18.9	377	31.1
M.C.	27	F	0.9	43	94.0	15.2	12.1	17.3	16.4	400	22.5
M.Co.	29	F	1.6	63	87.0	23.0	16.1	17.2	15.6	311	12.5
M.D.	22	F	1.2	50	85.5	17.8	12.8	21.9	20.7	371	20.5
R.F.	34	F	1.3	52	81.0	17.7	14.8	19.8	18.5	296	18.7
M.G.	40	F	1.4	56	116.4	27.1	18.7	19.2	17.8	330	11.8
J.J.	24	F	0.7	35	103.0	12.1	9.8	18.5	17.8	318	25.6
M.J.	37	F	1.2	49	117.8	24.1	19.9	18.0	16.8	264	12.5
O.J.	39	F	0.6	28	74.4	7.0	6.2	21.5	20.9	408	52.4
E.K.	35	F	0.9	46	94.1	15.5	10.0	15.2	14.3	274	18.3
B.M.	29	F	0.6	32	69.9	7.4	5.8	18.7	18.1	412	33.2
C.S.	40	F	1.1	42	100.0	17.6	15.7	22.0	20.9	346	20.9
A.P.	27	F	1.2	46	61.8	11.8	7.5	17.1	15.9	306	24.0
G.S.	31	M	0.8	41	84.0	11.6	9.6	30.5	29.7	365	43.9
H.S.	28	F	1.1	46	98.5	18.8	13.0	21.6	20.5	352	19.2
J.S.	35	M	0.9	43	73.1	10.5	8.2	26.2	25.3	330	41.5
L.S.	33	F	0.9	44	75.0	11.0	9.2	16.7	15.8	283	25.2
P.T.	17	F	0.9	39	105.0	13.5	10.0	14.8	14.0	219	18.3
A.W.	32	F	1.4	56	78.0	18.4	14.0	19.4	18.0	284	17.6
G.W.	34	F	0.8	38	96.6	12.1	10.0	19.9	19.1	319	27.3
T.W.	36	F	1.1	44	106.0	19.7	14.7	19.5	18.4	320	18.4
Mean			*1.0*	*44*	*90.7*	*15.9*	*11.9*	*19.4*	*18.3*	*327*	*23.3*
Standard Error			0.05	1.4	3.3	1.0	0.8	0.6	0.6	9	1.9
Range			0.6–1.6	28–63	60.0–124.0	7.0–25.0	5.7–19.9	14.7–30.5	13.7–29.7	219–412	11.5–52.4

* Thyroid/plasma iodide concentration ratio.
† TIS (cc.) ×K/60.

may reflect the severity of thyrotoxicosis only poorly, since the greatest discrepancies between the predicted and observed values were found in patients with the most marked disease.

Calculation of a predicted thyroidal uptake during any time interval is based upon the assumption that release of radioactive hormone does not occur during this period. It has been shown, however, that even in euthyroid patients, radioactive hormone is present in the plasma by the end of twenty-four hours (35). It might, therefore, be expected that even in the euthyroid patient, the predicted value would exceed the observed. Measurements of the butanol-extractable or "thyroxine" I^{131} in euthyroid patients show, however, that in such individuals the amount of radioactive hormone released from the gland within twenty-four hours after the administration of radioiodide is approximately 1 per cent of the administered dose, or less (35).

TABLE 2. GRAVES' DISEASE, UNTREATED

Patient	Age	Sex	TIS (liters)	T/P* ratio	K (% TIS/hr.)	T_T† (cc./min.)	C_T (cc./min.)	IDS (liters)	ETIDS (liters)	IDS/Kg. Body Wt. (cc.)	ITT (hrs.)
A.C.	24	F	2.3	90	115	43.6	35.2	18.5	16.2	370	7.1
J.C.	26	F	3.5	70	270	156.4	126.0	19.4	15.9	388	2.1
P.C.	17	F	3.4	96	414	232.5	160.1	15.3	11.9	214	1.1
A.F.	19	F	6.6	94	440	484.3	335.1	26.7	20.1	534	0.9
B.G.	32	F	5.1	85	180	153.3	113.3	29.8	24.7	618	3.2
D.H.	50	F	10.9	156	242	438.7	229.9	24.8	13.9	496	0.9
H.H.	35	M	4.5	70	589	446.0	338.5	21.5	17.0	378	0.8
W.H.	37	M	1.7	49	364	103.8	85.0	20.4	18.7	327	3.3
E.J.	32	F	2.4	81	115	46.5	34.9	18.4	16.0	321	6.6
J.K.	43	M	4.2	105	302	211.0	171.7	29.5	25.3	401	2.3
F.L.	34	F	4.2	70	493	342.1	269.6	24.8	20.6	399	1.2
C.M.	36	M	6.1	—	268	271.7	215.9	31.9	25.8	—	2.0
P.M.	17	F	9.0	129	1,210	1,822.0	1,274.1	21.0	16.5	375	0.4
M.N.	25	F	5.5	157	560	513.2	384.2	23.8	18.3	430	0.8
J.P.	58	M	2.8	81	501	237.0	196.4	24.3	21.5	461	1.7
M.R.	38	F	6.7	168	221	248.1	178.7	28.4	21.7	539	1.9
V.R.	32	F	2.0	50	403	135.5	114.0	16.4	14.4	254	2.0
M.S.	30	F	7.4	107	485	601.6	363.2	20.6	13.2	432	0.6
E.T.	24	F	18.2	455	363	1,100.3	572.0	34.0	15.8	608	0.5
G.T.	31	F	16.2	270	309	830.7	384.0	30.0	13.8	545	0.6
Mean			*6.1*	*125*	*392*	*420.9*	*279.0*	*24.0*	*18.1*	*426*	*2.0*
Standard error			1.0	22	53	94.9	61.0	1.2	0.9	26	0.4
Range			1.7–18.2	49–455	115–1,210	43.6–1,822.0	34.9–1,274.0	15.3–34.0	11.9–25.8	214–618	0.4–7.1

* Thyroid/plasma iodide concentration ratio.
† TIS (cc.) ×K/60.

The significance of the ultimate thyroidal uptake (I_U)

The physiologic significance of I_U exceeds that of the 24-hour thyroidal uptake of radioiodine. I_U represents the fractional conversion to hormone of all iodide entering the distribution space of this halide during any time interval.

When collection of radioiodine is incomplete at the end of twenty-four hours, the thyroid will have collected the observed percentage of iodide

TABLE 3. THE DISCREPANCY BETWEEN PREDICTED AND OBSERVED VALUES OF THE 24-HOUR RADIOIODINE UPTAKE

	Euthyroid	Hyperthyroid
No. of patients	29	10
Mean discrepancy*	0.5	−10.8
Range of discrepancy (% admin. dose)	(−2.1)–(3.9)	(−19.0)–(−2.6)

* Mean discrepancy between predicted and observed values of the 24-hour radioiodine uptake, as percentage of administered dose.

made available during this period, plus smaller percentages of iodide made available during the preceding days. This may be demonstrated mathematically.

During any 24-hour period, total hormone formation may be represented as the appropriate fractional conversion to hormone of available iodide from preceding days. According to equation 5,

$$I_H = \frac{K\ TIS\ I_{B_0}}{k} - \frac{K\ TIS\ I_{B_0}e^{-24k}}{k} + \frac{K\ TIS\ I_{B_0}e^{-24k}}{k} - \frac{K\ TIS\ I_{B_0}e^{-48k}}{k} + \frac{K\ TIS\ I_{B_0}e^{-48k}}{k} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad - \frac{K\ TIS\ I_{B_0}e^{-\infty k}}{k}$$

$$\Sigma I_H = \frac{K\ TIS\ I_{B_0}}{k} - \frac{K\ TIS\ I_{B_0}e^{-\infty k}}{k}.$$

Since

$$e^{-\infty k} = 0$$

$$\Sigma I_H = \frac{K\ TIS\ I_{B_0}}{k} = I_U.$$

Thyroidal iodide space

The thyroidal space of hyperthyroid patients in whom organic binding of iodine is inhibited has been found to be greater than normal (9). Measurement of its magnitude has been suggested as a possible test for the presence of this disease (8). The present studies emphasize the importance of the thyroidal content of inorganic iodide in determining the rate of manufacture of hormone. In all patients with hyperthyroidism, the thyroidal iodide space (mean, 6.1 liters) was greater than normal (mean, 1.0 liter). In neither group had hormone formation been inhibited by pharmacologic agents. This increase, although most marked in patients with the greatest degree of thyroidal enlargement, could not be explained solely by an increase in thyroidal size. Calculated values for the thyroid/plasma iodide concentration ratio, a measure of the ability of a unit mass of glandular tissue to concentrate iodide, revealed an increase in all patients with Graves' disease, except in 2 whose disease was mild. The normal gland appeared to concentrate iodide about 40-fold, whereas the mean ratio in hyperthyroid patients was 125. The largest TIS noted was 18.2 liters. In this

patient (E. T., Table 2), the thyroid-plasma iodide concentration ratio was approximately 450, and more than half the total body iodide was contained within the gland.

Little is known of the mechanism whereby the thyroid gland is enabled to maintain high concentration gradients of iodide. Recent observations suggest that the concentration of iodide is an active, energy-linked process (12). It would thus appear that in the hyperactive gland, the activity of these processes is increased.

Organic-binding rate

Measurements of the proportional rate of conversion of thyroidal iodide to organically-bound iodine have not heretofore been made. The present data indicate that in euthyroid individuals, approximately 90 per cent of the iodide contained within the gland is bound to protein per hour. A marked acceleration of organic binding was found in thyrotoxic patients (mean, 392 per cent/hour). Depletion of thyroidal iodide is prevented by the rapid entry of this anion from the plasma into the thyroid gland.

Since the organic-binding rate, unlike the thyroidal iodide space, is independent of thyroidal mass, and within limits of the total thyroidal iodide, the present findings suggest that in hyperthyroidism the intrathyroidal concentration of the enzyme or enzymes catalyzing the oxidation and binding of thyroidal iodide is increased. This finding is consistent with the observations of de Robertis *et al.* that, in the rat, thyroidal activation is associated with increased activity of peroxidase (36), an enzyme thought to be intimately concerned with the organic-binding process (37, 38).

Thyroidal transfer of plasma iodide

Unlike measurements of over-all radioiodine accumulation, determinations of the thyroidal clearance rate are thought to be independent of the rate of removal of iodide from the plasma. Thus, patient-to-patient variation in the rapidity of the removal of iodide by sites other than the thyroid gland do not directly influence the clearance rate. Several recent reports (39, 40) dealing with thyroidal clearances in normal and hyperthyroid patients have presented data derived according to the conventional clearance formula, in which

$$\text{Clearance} = \frac{\text{rate of change of thyroidal } I^{131}}{\text{plasma } I^{131} \text{ concentration}}. \qquad 22$$

Clearance, so defined, will be designated "C_T." From equation 8, the rate of change of thyroidal I^{131} (slope of the radioiodine uptake curve),

$$dI_T/dt = TIS\ I_{B_0}e^{-kt}(K - k).$$

From equations 1, 8 and 22,

$$C_T = \frac{TIS\ I_{B_0}e^{-kt}(K - k)}{I_{B_0}e^{-kt}}$$

$$= TIS\ (K - k). \qquad 23$$

When K is less than k, *i. e.*, when the rate of loss of radioiodide from the plasma, and hence from the thyroidal iodide space, is greater than its rate of incorporation into hormonal iodine, the slope of the iodine uptake curve is negative, and C_T is also negative. Figure 2 demonstrates that hormonal radioiodine may be formed, and iodide may truly be cleared from the plasma when C_T is negative. Measurements of C_T have little significance under these circumstances, as recognized by the authors of the reports cited. Since the slope of the radioiodine uptake curve is dependent in part on k (the rate of removal of radioiodide from the plasma), it must, in turn, be influenced by the renal excretion of iodide. Thyroidal clearances, calculated on the basis of the rate of change of total thyroidal I^{131}, are thus not entirely independent of the rate of excretion of this halide by the kidneys.

The present method makes possible calculation of the rate of transfer of plasma iodide to hormonal iodine, a value which is not derived from the slope of the uptake curve and which is therefore independent of changes in the radioactivity of thyroidal iodide. In order to differentiate this function from conventionally determined clearance rates, it will be designated the "thyroidal transfer rate" (T_T).

From equation 4, the rate of change of hormonal radioiodine,

$$dI_H/dt = K\ TIS\ I_{B_0}e^{-kt}.$$

Therefore,

$$T_T = \frac{K\ TIS\ I_{B_0}e^{-kt}}{I_{B_0}e^{-kt}}$$

$$= K\ TIS. \qquad 24$$

Equation 9 is now seen to be identical to the conventional formula for the removal of a material with an exponential disappearance rate, in which the factor, TIS K represents the thyroidal transfer rate (41). T_T, thus defined is never negative and approaches zero only as TIS or K, or both, become vanishingly small.

The mean thyroidal transfer rate in euthyroid individuals was found to be 15.9 cc./min. (range, 7.0–25.0). This is equivalent to a total daily transfer of approximately 22.9 liters. This value is remarkably close to that predicted by Riggs on the basis of the average normal iodine intake and excretion (42). The thyroidal transfer rate in patients with Graves' disease was

found to be markedly increased (mean, 420.9 cc./min.; range, 43.6–1,822.0).

Values of C_T calculated in the same groups of patients, revealed a mean of 11.9 cc./min. in euthyroid and 279.0 cc./min. in hyperthyroid patients. T_T always exceeds C_T by the factor, TIS k, which represents the rate of loss of radioiodide from the thyroidal iodide space. It is not surprising therefore that the discrepancy between T_T and C_T is smaller in normal than in hyperthyroid patients, in whom both TIS and k are usually increased.

The most rapid transfer rate noted was 1,822 cc./minute. The transfer rate delimits the rate of blood flow to the gland, assuming complete extraction of perfusing iodide. The exceedingly large rates noted in patients with severe thyrotoxicosis suggest that a considerable fraction of the increased cardiac output associated with this disease may be apportioned to the gland itself.

The increased iodine-accumulative function found in Graves' disease generally results from increases in both TIS and the binding rate. The data, however, reveal no clear correlation in the individual hyperthyroid patient, between the magnitude of the TIS and that of the binding rate. In some patients, an increase in the TIS was the preponderant cause of the increased function of the gland; in others, the chief cause was an accelerated binding rate. In most patients, however, both factors were increased, although not necessarily to the same extent.

Iodide distribution space

In confirmation of previous reports (22, 29), the iodide distribution space of hyperthyroid patients was found usually to exceed that of normal individuals, whether expressed as a total space or as a fraction of the body weight. If the iodide in the extracellular fluid is indeed in rapid exchange with that in the thyroidal iodide space, the latter will be measured as a portion of the total volume of distribution of this halide. The mean extrathyroidal iodide distribution space (ETIDS), calculated by correcting the total iodide space for its intrathyroidal component, was virtually identical in hyperthyroid and euthyroid patients. The extrathyroidal iodide distribution space of hyperthyroid patients, obtained by measurement of thyroidal and urinary radioiodine collection and plasma radioiodide concentration, has been reported to be greater than normal (43). The close agreement noted in the present study between values found in normal and hyperthyroid subjects may be fortuitous, since, in the hyperthyroid patients herein reported, plasma radioiodide concentration curves were obtained sooner after the injection of I^{131} than in normal subjects. Thus the radioiodide may not have been as nearly completely distributed in hyperthyroid as in euthyroid patients.

Equation 9 suggests that the eventual uptake of radioiodine is dependent

in part upon the volume of distribution of iodide. It can be demonstrated, however, that this is not the case. The total plasma radioiodide clearance rate is the sum of the thyroidal and the extrathyroidal removal rates, and may be represented thus where C_{ET} = extrathyroidal iodide clearance rate

$$100k/I_{B_0} = K\ TIS + C_{ET}$$

$$k = I_{B_0}\ (K\ TIS + C_{ET})/100 \qquad 25$$

$$dk/dI_{B_0} = (K\ TIS + C_{ET})/100. \qquad 26$$

Equation 26 demonstrates that as I_{B_0} changes, k changes proportionately, so that from equation 9, I_U remains unchanged. Furthermore, since from equations 9 and 26

$$I_U = \frac{T_T I_{B_0}}{k}$$

from equation 25,

$$I_U = \frac{T_T I_{B_0}}{I_{B_0}(T_T + C_{ET})/100}$$

$$= \frac{100\ T_T}{T_T + C_{ET}}. \qquad 27$$

The thyroidal uptake of radioactive iodine is thus representative of the fractional contribution of the thyroid gland to the total rate of removal of iodide from its distribution compartment. Equation 27 reveals that when the thyroidal transfer rate is already elevated, further marked increases in this function are associated with only small increases in radioiodine uptake. This consideration provides further evidence that the 24-hour radioiodine uptake is inferior to determination of thyroidal transfer rate as a measure of degree of thyroidal hyperactivity.

One-hour I^{131} uptake

The thyroidal uptake of radioactive iodine, observed one hour after the administration of a tracer dose of radioactive iodine, has been proposed as a convenient method for distinguishing between patients with normal and increased thyroidal function, and for obviating some of the foregoing difficulties inherent in the interpretation of the 24-hour radioiodine uptake (33, 44, 45). The present studies have afforded an opportunity of correlating the one-hour radioactive iodine uptake with the thyroidal transfer rate. As pointed out by Myant *et al.* (43), plasma concentrations of radioiodide during the first hour after its administration do not differ markedly between normal and thyrotoxic subjects. Since the uptake of radioiodine is dependent upon the thyroidal transfer rate and the plasma radioiodide

concentration, a good correlation between the one-hour uptake and the thyroidal transfer rate could be predicted, and has been found (Fig. 5). A rather clear demarcation between normal and thyrotoxic patients was observed. The mean one-hour uptake in euthyroid individuals was found to be 9.0 per cent of the administered dose (std. dev., 2.8 per cent). It would

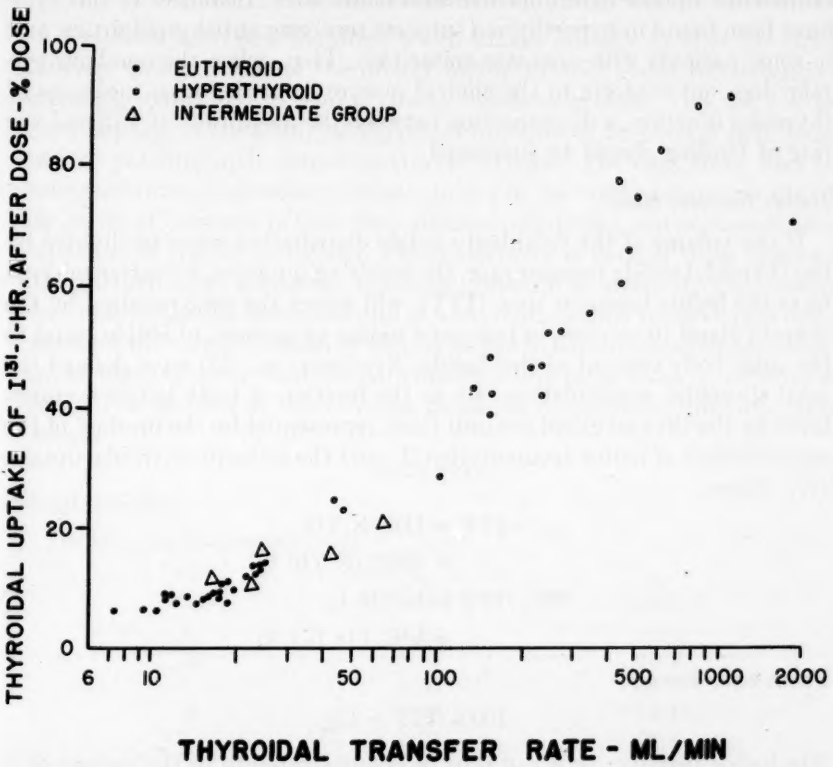

FIG. 5. The relationship between the one-hour thyroidal uptake of I^{131} and the thyroidal iodide transfer rate. Logarithmic plot is employed in order to conserve space. On cartesian coordinates, as transfer rate increases, the one-hour uptake asymptotically approaches 100 per cent. Intermediate group consists of patients in whom the results of all studies (including BMR, PBI, and butanol-extractable I^{131}) ranged in the borderline between euthyroidism and hyperthyroidism.

be anticipated, therefore, that approximately 90 per cent of euthyroid individuals would demonstrate one-hour uptakes between 4.4 and 14.6 per cent of the administered dose. In the present studies, none of the definitely thyrotoxic patients displayed values within this range.

Analysis of the one-hour thyroidal radioactive iodine uptake according

to the concepts herein described reveals that this measure is the resultant of both the rapid initial uptake of inorganic iodide, I_{C_0}, and the subsequent slope of the iodine uptake curve. When I_{C_0} is large and the slope of the uptake curve is negative or even shallow, although positive, the one-hour uptake may not correlate well with the thyroidal clearance rate, or with the radioiodine uptake determined several hours later. Instances of this type have been found in hyperthyroid subjects receiving antithyroid drugs, and in some patients with nontoxic goiter (46). Thus, when the one-hour uptake does not conform to the clinical picture or to the other measures of thyroidal function, a disproportion between the magnitude of TIS and the rate of binding should be suspected.

Iodide turnover time

If the volume of the total body iodide distribution space be divided by the thyroidal iodide transfer rate, the resulting quotient, hereafter referred to as the iodide turnover time (ITT), will reflect the time required by the thyroid gland to convert to hormonal iodine an amount of iodide equal to the total body content of this halide. Keating *et al.* (22) have defined the total thyroidal accumulation rate as the fraction of body iodide accumulated by the thyroid gland per unit time, represented by the product of the rate constant of iodine accumulation (k) and the ultimate thyroidal uptake (I_U). Since,

$$\begin{aligned} ITT &= IDS/K\ TIS \\ &= 100\%/K\ TIS\ I_{B_0} \\ 100\%/ITT &= K\ TIS\ I_{B_0} \\ &= k(K\ TIS\ I_{B_0})/k. \end{aligned}$$

From equation 9,

$$100\%/ITT = kI_U.$$

The iodide turnover time can thus be demonstrated to be the reciprocal of the thyroidal accumulation rate.

The average value of ITT in normal individuals has been found to be twenty-four hours. If the average normal concentration of iodide in the plasma be 3 μgm./liter (42) and the iodide distribution space be 19 liters, then 57 μgm. of iodide will be turned over by the thyroid in the 24-hour period. This amount is considerably less than the average daily dietary intake of iodide (42). In hyperthyroid patients, the ITT averaged 2.0 hours. The average concentration of iodide in the plasma of hyperthyroid patients appears to be at least as great as normal (46). Assuming a volume of distribution of 24 liters, at least 72 μgm. of iodide will be turned over each two hours, or 864 μgm. each day. Since this value greatly exceeds the average

daily dietary intake of iodide, depletion of body iodide would be expected to occur, were not an additional source of iodide available. Iodide released from thyroid hormone as a consequence of its peripheral utilization provides the source for the increased demands for iodide of the thyrotoxic patient (46).

SUMMARY

A method has been described which makes possible in man the simultaneous measurement of the ability of the thyroid gland to concentrate inorganic iodide and to bind thyroidal iodide to protein. This method has been applied to the study of thyroidal function of 28 normal individuals and 20 patients with untreated Graves' disease. The data show that the increased rate of hormone synthesis found in the latter group was usually the result of increases in both the iodide-concentrating and protein-binding capacities of the thyroid gland. The importance of each of these factors in the production of abnormal thyroidal function is stressed. The method also makes possible determination of a thyroidal iodide transfer rate which measures the rate of transfer of plasma iodide to hormonal iodine and which can be applied to the study of patients receiving large doses of antithyroid drugs. The relation of this to other measures of thyroidal function is discussed.

APPENDIX

Sample calculation

The following data are obtained:

t (hours)	I_B (% dose/L.)	I_T (% dose)
1	4.20	10.6
$1\frac{1}{2}$	3.85	12.2
2	3.50	13.7
$2\frac{1}{2}$	3.20	15.1
3	2.93	16.4

By extrapolation, $I_{B_0} = 5.06$.

Two points in time, $t = 1\frac{1}{2}$ and $t = 3$, are selected.

$$\frac{I_U - 12.2}{I_U - 16.4} = \frac{3.85}{2.93}$$

$$0.92\ I_U = 27.39$$

$$I_U = 29.8 \text{ (\% dose)}$$

$$I_{R_0} = (29.8 - 12.2) \times 5.06/3.85 = 23.1 \text{ (\% dose)}$$

$$I_{C_0} = 29.8 - 23.1 = 6.7 \text{ (\% dose)}$$

$$TIS = 6.7/5.06 = 1.324 \text{ (liters)}$$

$$e^{-1k} = 4.20/5.06 = 0.830$$

$$k = 0.186 \text{ (fraction/hour)}$$

$$K = 0.186 \times 29.8/6.7 = 0.827 \text{ (fraction/hour)}$$
$$T_T = 0.827 \times 1.324 = 1.095 \text{ (liters/hour)}$$
$$IDS = 100/5.06 = 19.8 \text{ (liters)}$$
$$ETIDS = 19.8 - 1.3 = 18.5 \text{ (liters)}$$
$$ITT = 19.8/1.095 = 18.1 \text{ (hours)}$$

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Teaching Clinic

ROLE OF THE NEUROHYPOPHYSEAL-ANTIDIURETIC-HORMONE-RENAL SYSTEM IN EVERYDAY CLINICAL MEDICINE*

THE present clinic is designed to illustrate the practical value of information concerning the homeostatic functions of endocrine systems in the management of endocrinologically normal patients presenting with common types of illness.

The first section of the clinic reviews briefly the salient functional characteristics of the neurohypophyseal-antidiuretic hormone (ADH)-renal system. Subsequent sections present illustrations of the manner in which this system has been observed to function in common clinical situations.

Role of normal neurohypophyseal-antidiuretic hormone (ADH)-renal system in homeostasis of serum water concentration

It will be remembered that this system is comprised of hypothalamic receptors which react to stimulate release of ADH when the water concentration of serum is less than about 3.6, and to discontinue ADH release when water concentration is more than about 3.6 cc. per milliosmol of total solute[1] (1). As shown in the left-hand section of Figure 1, when maximum ADH secretion is called forth by water deprivation, urine water concentration is reduced to a physiologic minimum of about 0.7 cc. per m-osm. Contrariwise, when ADH secretion is suppressed by administration of a generous load of water to a normally hydrated individual, urine water concentration rises to physiologic maximal levels of approximately 10 cc. per m-osm. The rapidity with which the system responds to either of these stimuli when the change in serum water concentration occurs abruptly is illus-

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[1] The ratio of water to solutes in serum indicated here, namely, 3.6 cc. per milliosmol, is higher than that given in an earlier publication (1), namely, 3.2 cc. per milliosmol. The difference is due to alterations in the method of expressing results (2).

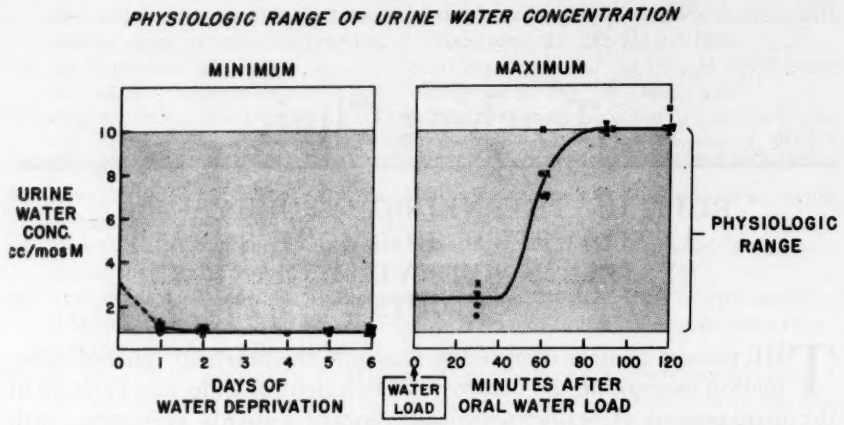

FIG. 1. Description of usual upper and lower limits of renal ability to vary urine water concentration. The minimum data are from unpublished observations of Gamble, Butler and Talbot; the maximum are from unpublished data of Crawford and Schoen.

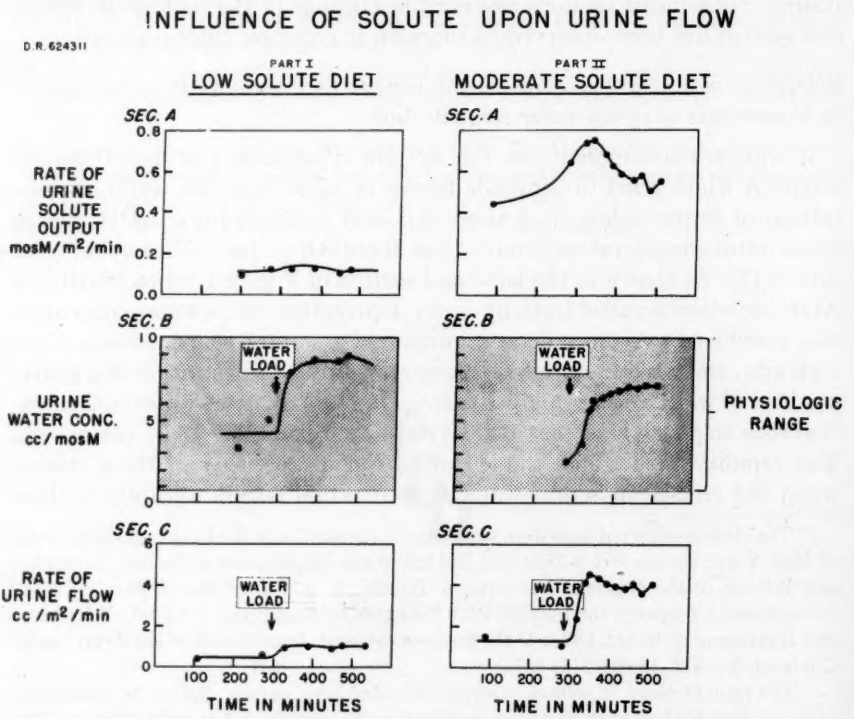

FIG. 2. Limiting influence of urine solute output upon maximum attainable urine volume.

trated by the data of the right-hand section of Figure 1. This range in bodily capacity to vary urine water concentration in accordance with need normally enables the organism to keep plasma water concentration between 3.5 and 3.7 cc. per m-osm.

The ability of this system to maintain homeostasis of body water concentration under conditions of varying water intake is determined to a considerable extent by solute intake and output. This is illustrated in

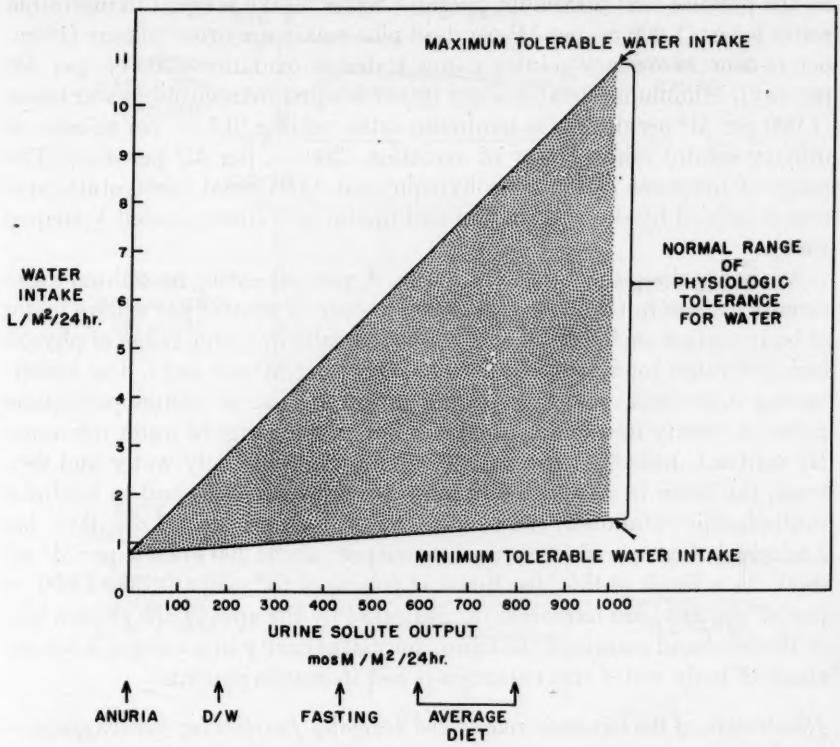

FIG. 3. Influence of urine solute output on the body's normal range of physiologic tolerance for water.

Figure 2, which gives information concerning the effects of urinary solute output upon renal ability to excrete water. The subject of these studies was first given a diet of low solute residue (left-hand sections) and second, a diet of moderate solute residue (right-hand sections). As a consequence of his dietary regimens he was excreting solutes in the urine at a rate of about 0.1 m-osm. per minute in the first instance (left section A) and at about 0.6 m-osm. per minute in the second instance (right section A). As shown in Section B, he was given a load of water sufficient to

result in both instances in the formation of maximally dilute urine (in this patient, urine water concentration was about 8 cc. per m-osm). Rate of urine flow, which is the product of solute output times water concentration, was maximal at about 1 cc. per minute during the low solute and at about 4 cc. per minute during the moderate solute regimen.

The effects of the foregoing variables upon total bodily tolerance for water are set forth in approximate terms in Figure 3. This diagram is based on the premise that maximum tolerable water intake is equal to insensible water losses (1,000 cc. per M^2 per day) plus maximum urine volume (10 cc. per m-osm. of urinary solute) minus water of oxidation (200 cc. per M^2 per day). Minimum tolerable water intake is equal to insensible water losses (1,000 per M^2 per day) plus minimum urine volume (0.7 cc. per m-osm. of urinary solute) minus water of oxidation (200 cc. per M^2 per day). The range of tolerance of the neurohypophyseal-ADH-renal homeostatic system is defined by these maximum and minimum values (shaded V-shaped zone).

As shown along the bottom of Figure 3, persons eating an ordinary diet usually excrete in the urine 600 to 800 m-osm. of solutes per square meter of body surface each day. Such a regimen results in a wide range of physiologic tolerance for water (1,200 to 8,000 cc. per M^2 per day). The ketotic fasting individual, who excretes about 450 m-osm. of solutes per square meter in twenty-four hours, also has a fairly wide range of water tolerance. By contrast, note that the patient who is receiving only water and dextrose, the latter in amounts sufficient to eliminate ketosis and to minimize protoplasmic catabolism (75 to 100 Gm. of dextrose per M^2 per day), has a relatively low rate of urinary solute output (about 200 m-osm. per M^2 per day). As a result of this, the limits of tolerance for water (1,000–2,800 cc. per M^2 per day) are narrowed. As indicated by the apex of the shaded area at the left-hand margin of the figure, normal capacity to accomplish homeostasis of body water concentration is lost in anuric patients.

Illustration of the dynamic role of the normally functioning neurohypophyseal-ADH-renal system in the repair and maintenance of body water concentration in a dehydrated patient

Figure 4 presents observations made on a four-month-old infant who had become dehydrated as a result of diarrhea. Section A gives information concerning serum water concentration. It is evident that serum water concentration was subnormal initially. After a slight transient decrease,[2] it rose to normal levels by the twelfth hour of therapy and remained within nor-

[2] Experience with other patients indicates that this initial fall in serum water concentration was due to a transient increase in serum dextrose concentration at the onset of dextrose administration.

mal limits thereafter. Section B shows that urine water concentration was maintained close to physiologic minimum levels during the period when serum water concentration was subnormal and then rose to higher levels, where it remained after repair of serum water deficits had been accomplished. Section C shows the rate of intravenous infusion of a multiple electrolyte-dextrose solution.[3] During the first twenty-four hours, the volume infused (about 3.3 liters per M^2 per day) was considerably greater

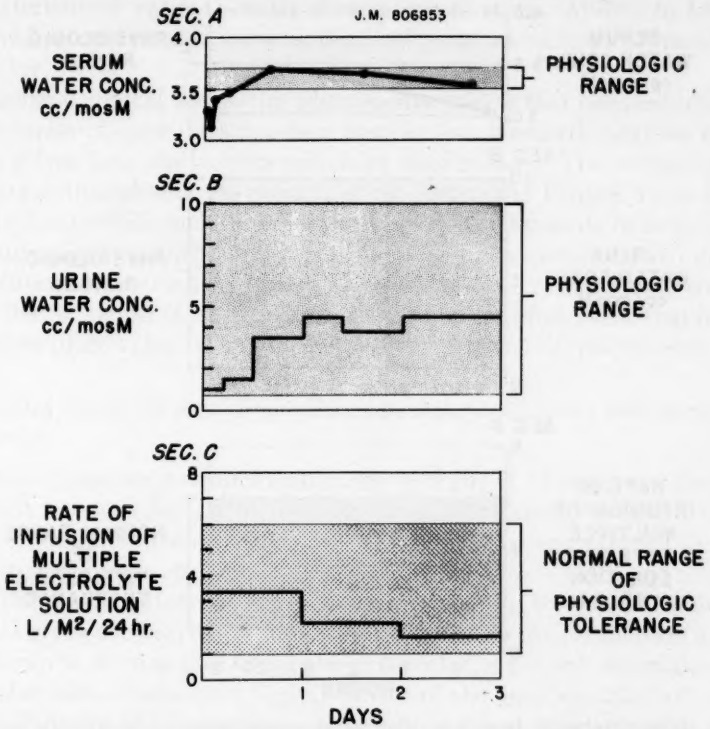

FIG. 4. Example of role played by normally functioning neurohypophyseal-ADH-renal system in restoration of serum water concentration to normal limits.

than the 1 liter per square meter per day needed to provide a normal individual with his basic water needs, and appreciably less than the largest amount of this particular solution which such an individual should be able

[3] The multiple electrolyte solution used here contained 40 mEq. of Na, 35 mEq. of K, 40 mEq. of Cl, 20 mEq. of lactate and 15 mEq. of phosphate, or a total of 150 m-osm. of electrolyte solutes per liter. It also contained 50 Gm. of dextrose per liter. The body's physiologic range of tolerance for this solution as represented by the shaded zone of Section C, Figure 5, has been discussed in a previous communication (3).

to eliminate completely without upsetting body economy (about 6 liters per M^2 per day (3)). During the second and third days the rate of infusion was diminished to 2.0 and 1.5 liters per M^2 per day, respectively.

Viewed as a whole, these data illustrate the perfection with which the normally functioning neurohypophyseal-ADH-renal system accomplishes homeostases of serum water concentration when provided with reasonably

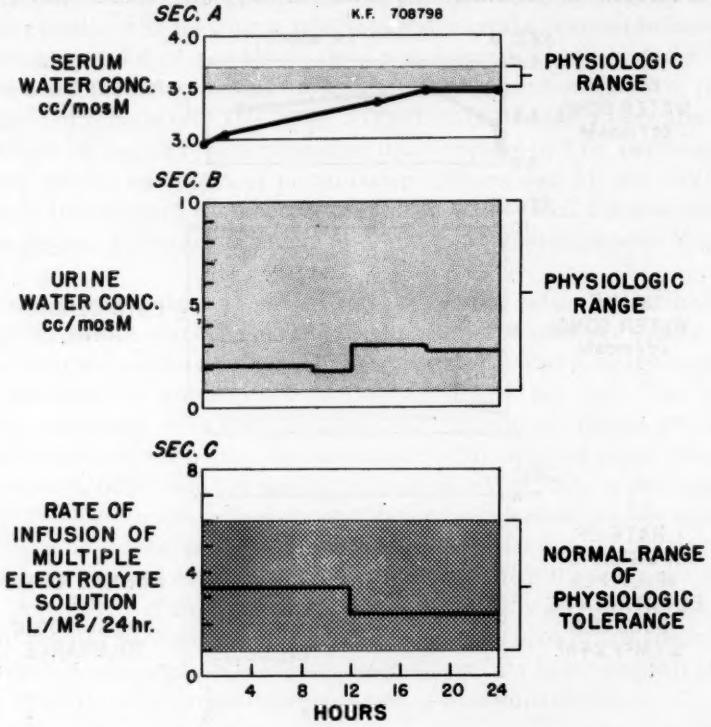

FIG. 5. Loss of capacity to reduce urine water concentration to physiologic minimum levels in accordance with homeostatic needs, due to severe stress.

generous, but physiologically tolerable amounts of water and solutes. The sections which follow are concerned with the effects of factors other than neurohypophyseal disease upon the functional behavior of this homeostatic system.

Temporary reduction in capacity of neurohypophyseal-ADH-renal system to conserve water during periods of severe stress

Figure 5 sets forth measurements obtained on a 15-year-old girl with marked diabetic ketoacidosis, as shown by a serum pH of 6.9 and a serum

bicarbonate concentration of 4.9 mEq. per liter. It may be seen in section A that her initial serum water concentration was markedly reduced below normal levels. Section B shows that urine passed during the first eight hours of observation had a water concentration of 2 cc. per m-osm. This is twice the concentration value found for the patient of Figure 4 and about three times the physiologic minimum of 0.7 cc. per m-osm. described in Figure 1. Such a partial loss of ability to conserve water at the kidney is characteristic of patients under chronic severe stress. Ability to form a maximally concentrated urine is regained upon restoration of metabolic normalcy.

In general clinical terms, this phenomenon means that persons suffering severe stress of some duration may have an extraordinary need for water which is free from electrolytes and other fixed solutes.[4] The multiple electrolyte solution used in the therapy of the patients of Figures 5 and 6 was designed to provide such "free" water in moderate amounts in association with appreciable quantities of electrolyte. This was accomplished by giving the solution a water concentration (7 cc. per m-osm. of electrolyte-solute) twice that of serum (3.5 cc. per m-osm.) and about three times that of the minimum urine value for a stressed patient (2 to 2.5 cc. per m-osm.) (3).

Temporary limitation in ability to eliminate water due to ether and morphine-type drugs

Figure 6 presents measurements made on a girl of 12 years who had her appendix removed during the second hour of observation. Section A shows that her serum water concentration was normal preoperatively. During the first thirty hours she received ether, morphine sulfate (MS) and meperidine (Demerol) in standard doses as indicated along the top of Section B and was given the previously described multiple electrolyte solution at the rate shown in Section C. During this interval, the patient complained of headaches and in retrospect had a number of the manifestations of water excess. The Demerol was given in an effort to relieve her headaches. The true nature of her headaches was not recognized until the thirtieth hour, when she suffered a generalized convulsion. At this time a diagnosis of water intoxication was made. Fluid administration was discontinued immediately and the patient made an uneventful recovery.

An explanation for this individual's difficulties is provided by the urine water concentration data of Section B. The interrupted line indicates the values for urine water concentration required for this patient to maintain serum water concentration within normal limits under the conditions of

[4] Dextrose is not considered a fixed solute because it is metabolized to water and carbon dioxide.

water plus solute intake and output actually pertaining during the interval shown. It is seen that these theoretical values lay near the center of the physiologic range. The solid line indicates that the patient's measured urine water concentration values were much lower than the ideal represented by the interrupted line and thus were grossly out of keeping with homeostatic needs; hence, the described pathologic rise in serum water concentration.

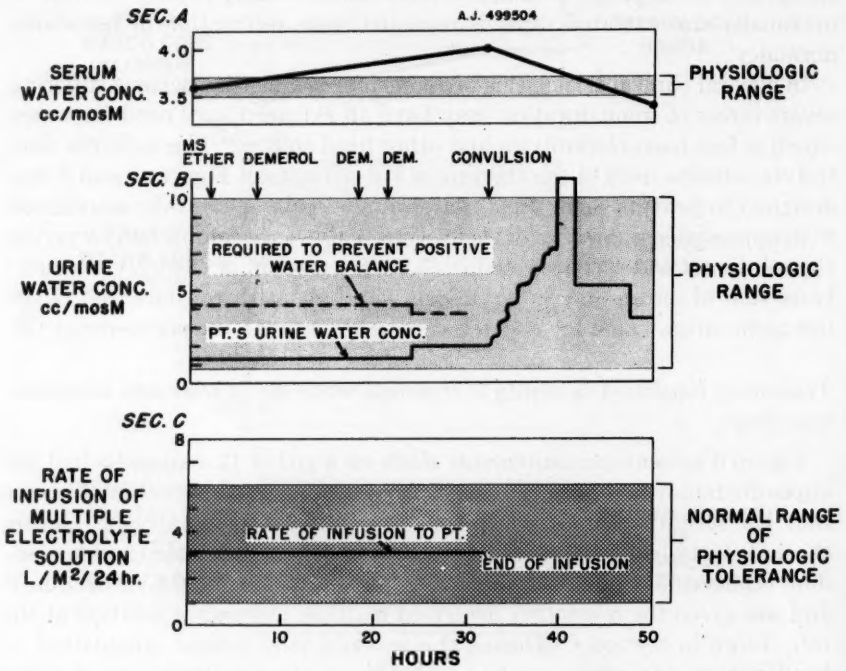

FIG. 6. Loss of capacity to increase urine water concentration to physiologically maximum levels in accordance with homeostatic needs, due to ether and morphine-type drugs.

Urine voided during the eight-hour period following the convulsion was lost. Urine collected subsequently was, however, found to be of physiologic maximal water concentration. This demonstrated that the patient had a normal capacity to increase urine water concentration in accordance with homeostatic need, after the influence of anesthesia and morphine-type drugs had worn off.

The course of events noted in this patient is in keeping with the fact that ether, being a solute of significant osmotic activity at therapeutic con-

centration, acts to lower serum water concentration during the induction of anesthesia (4). This apparently results in release of massive amounts of ADH from the posterior pituitary body. In addition, morphine and its derivatives potentiate and prolong the action of ADH thus released. It follows that patients who have been given these drugs are apt to show a loss of normal capacity to form a urine of high water concentration during operation and for several hours postoperatively. Risk of inducing water

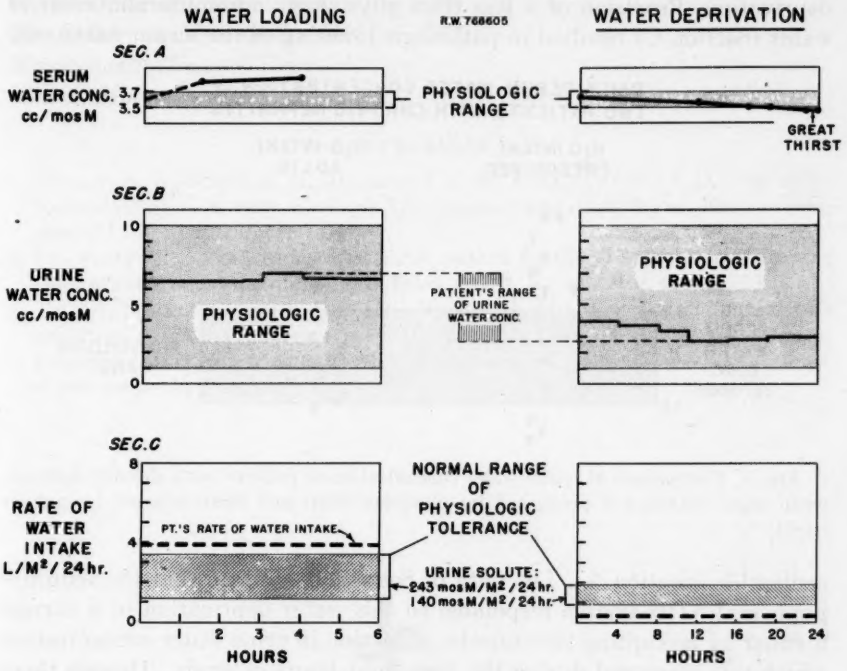

FIG. 7. Partial loss of capacity to vary urine water concentration in accordance with homeostatic need, due to renal disease.

intoxication during this period of reduced homeostatic capacity can largely be avoided by restricting the rate of infusion of fluids to approximately 60 cc. per M^2 per hour (100 cc. per adult patient per hour).[5]

Permanent limitation in capacity for water homeostasis due to impairment of renal function

Figure 7 summarizes studies carried out on a 20-year-old male patient with marked impairment of renal function, manifested by a urea clearance

[5] Though risk of water intoxication can be eliminated by employing isotonic saline solution, use of this solution is apt to result in sodium retention and edema formation.

of 5 per cent of normal. The left-hand diagrams show that administration of water at a physiologically excessive rate for this patient (Section C) caused serum water concentration to rise to abnormally high levels (Section A). Under these circumstances the normal person forms a urine having a water concentration of at least 10 cc. per m-osm. (Fig. 1); but the present patient excreted urine containing only 6.5 cc. per m-osm.

The right-hand diagrams indicate his response to a test period of water deprivation. Provision of a less than physiologic minimum allotment of water (Section C) resulted in pathologic lowering of the serum water con-

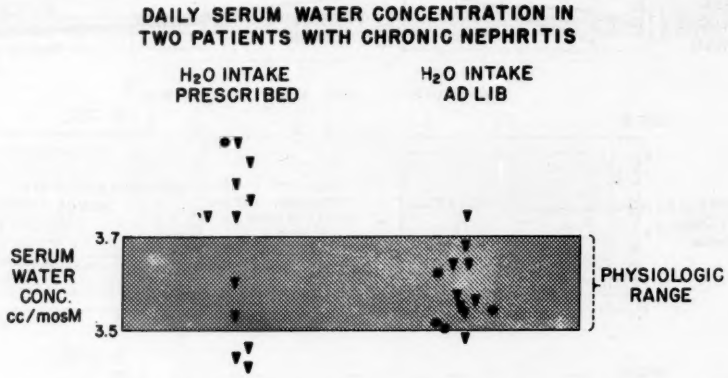

FIG. 8. Comparison of serum water concentration in patients with chronic nephritis, when water intake was prescribed by physician (left) and when selected by patient (right).

centration (Section A). The data of Section B indicate that his neurohypophyseal-ADH-system responded to this water deprivation in a normal manner by prompting the stepwise reduction in urine water concentration which was measured during the first eight hours of study. Though there was need for full physiologic water conservation by the kidney during the latter hours of the study, renal damage prevented this patient from reducing his urine concentration below 2.8 cc. per m-osm.

Such reduction in the functional capacity of the neurohypophyseal-ADH-renal system exists in a large number of patients with severe impairment of renal function. If such individuals are to avoid the manifestations characteristic of water excess and of water lack, their intake of water must lie within a relatively narrow range. Fortunately, the patient often is able, with the aid of his thirst mechanism, to sense his total water needs with considerable accuracy. This is illustrated by the data of Figure 8, which shows how 2 nephritic patients were better able to judge their water needs and thereby maintain essentially normal serum water concentration val-

ues than were physicians who attempted to maintain the body water concentration of these patients within normal limits by prescribing fluid intakes.

GERALD A. KERRIGAN, M.D.
NATHAN B. TALBOT, M.D.
JOHN D. CRAWFORD, M.D.

The Department of Pediatrics,
Harvard Medical School, and
The Children's Medical Service,
Massachusetts General Hospital,
Boston, Massachusetts

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Editorial

DYNAMIC ENDOCRINOLOGY IN EVERYDAY CLINICAL MEDICINE*

THE Selective Service statistics of the second world war show that primary endocrinopathies accounted for about 1 per cent of the total number of rejections for military service (1). In the year 1950 at the Massachusetts General Hospital, 1.4 per cent of the total out-patient attendance of approximately 200,000 patient visits was for care of thyroid disorders, 1.2 per cent for treatment of diabetes mellitus and 0.5 per cent for diagnosis and management of other primary endocrinopathies (2). These data are in keeping with the commonly held impression that primary endocrine disorders comprise but a small fraction of the medical problems encountered in general clinical practice.

In contrast, it is of interest to consider the frequency with which intravenous infusions are employed in hospitals today and the endocrinologic implications of this practice. In 1950, approximately 17,000 persons were admitted as in-patients to the Massachusetts General Hospital for a total of about 240,000 patient days (2). During this interval approximately 65,000 liters of parenteral fluid were dispensed. This amounts to about 3.8 liters of fluid per patient admitted, or 1 liter every four hospital days. If these figures are representative, they would suggest that enormous volumes of fluid are administered parenterally to patients in this country every year.

The response of patients to parenteral fluid therapy is governed to a considerable extent by endocrine-regulated homeostatic systems[1]. For instance, the water infused must be retained or rejected by the neurohypophyseal-renal system in accordance with physiologic needs, or the patient is liable to become either dehydrated or water intoxicated. Likewise sodium given parenterally must be stored or excreted according to bodily needs, if edema or cellular sodium intoxication on the one hand and patho-

* The points of view expressed here are based in part on studies supported by the Commonwealth Fund of New York; Mead Johnson and Company; and Grants No. G-2831 and H-1529 of the Department of Health, Education and Welfare of the National Institutes of Health, U. S. Public Health Service.

[1] Basic information concerning the functional characteristics of these systems, together with references, has been set forth in a previous communication (3).

logic shrinkage of extracellular fluid volume on the other are to be avoided. Sodium regulation appears to be under the control of a complex homeostatic system which includes the hypothalamus, anterior pituitary, adrenal cortices, perhaps the adrenal medullae, the kidney, gut and sweat glands. Bodily potassium homeostasis seems also to be determined by this same system.

Phosphate is another electrolyte which is being added to intravenous solutions with increasing frequency. Administration of reasonable quantities of this ion usually is safe because the parathyroid-renal homeostatic system normally acts to prevent hyperphosphatemia by increasing phosphaturia when phosphate entering the body is in excess of current requirements. Likewise, blood glucose homeostasis is dependent upon the integrated functioning of the anterior pituitary, adrenal cortices, adrenal medullae and pancreatic islets. Together, they attempt to keep blood glucose concentration within physiologic limits under the widely varying rates of carbohydrate intake which are apt to obtain before, during and after institution of intravenous fluid treatment.

Although something is known, much has yet to be learned about the precise nature of the physiologic variables to which each of these systems is intended to react, the locations of the organs which sense changes in each of the variables mentioned, the normal reactive "setting" of the various sensing elements, the mechanisms by which the endocrine-homeostatic systems accomplish their individual purposes, the time needed for these reactions to take place, and the limits of capacity of each system to cope successfully with its particular responsibility. We also have inadequate knowledge concerning the effects of changes in the activity status of one system upon the functional performance of others, the extent to which drugs and certain diseases may modify the behavior or limit the capacity of certain of these homeostatic systems and the effects of over-reaching the capacity of any of these homeostatic mechanisms (see Teaching Clinic).

Endocrinologic considerations are important not only in the design of parenteral fluid therapy but also to the understanding of certain situations in which clinical manifestations are the product of a normal, endocrine-mediated homeostatic reaction to an abnormal body circumstance. A classically described example of this type of phenomenon is rickets due to base-losing nephritis. Here, in a physiologic effort to overcome the tendency to hypocalcemia occasioned by the renal tubular defect, the parathyroids release calcium from skeletal stores with resultant osteomalacia or rickets. Incidentally, it may be emphasized that these patients are euparathyroid rather than hyperparathyroid as sometimes stated (3). That is, the increase in parathyroid activity observed in such patients is normal for the abnormal circumstance.

Another example of this type of phenomenon may be seen in certain nephrotic patients whose edema appears to be homeostatically determined and not the obligatory result of an absolute inability to excrete sodium and water at rates sufficient to eliminate the edema. Since some edematous nephrotic children can vary their urinary sodium and water excretion in response to variations in intake, in an essentially normal manner (4), their edema appears to be facultatively determined. Observations of this sort make one wonder how many other conditions there may be, which at first glance seem to be static disturbances but actually are the product of a normal dynamic homeostatic reaction. Evidently, under certain circumstances, homeostatic adjustments in one area may result in pathologic developments elsewhere.

If the statistics quoted here are fairly representative and the remarks which accompany them are valid, it would appear that knowledge concerning dynamic or functional endocrinology should be widely useful. Paradoxically, informal survey reveals that only approximately 7 per cent of the papers published in the Journal of Clinical Endocrinology & Metabolism and in Endocrinology over the past ten years have been concerned primarily with these homeostatic aspects of endocrinology. Is this to be considered an indication that these metabolic phenomena are not considered within the realm of endocrinology by those most skilled in this discipline or are opportunities for exploration in this field not as yet being fully realized?

NATHAN B. TALBOT, M.D.
JOHN D. CRAWFORD, M.D.
GERALD KERRIGAN, M.D.

The Children's Medical Service,
Massachusetts General Hospital, and
Department of Pediatrics,
Harvard Medical School,
Boston, Massachusetts

REFERENCES

1. ROWNTREE, L. G.: Endocrine diseases as revealed by 13,000,000 examinations of registrants, *J. Clin. Endocrinol.* **4**: 545, 1944.
2. Trustees of the Massachusetts General Hospital: One Hundred and Thirty-Seventh Annual Report, 1950.
3. TALBOT, N. B.; SOBEL, E. H.; MCARTHUR, J. W., and CRAWFORD, J. D.: Functional Endocrinology From Birth Through Adolescence. Cambridge, Mass., Harvard University Press, 1952.
4. COOK, C. D.; CRAWFORD, J. D.; CORSA, L., and TALBOT, N. B.: Studies of sodium homeostasis by normal and by nephrotic individuals, *in* Third Annual Conference on the Nephrotic Syndrome, Dec. 13–15, 1951, edited by J. Metcoff. Published under the Auspices of the National Nephrosis Foundation, Inc., New York.

The Endocrine Society.

THE 1955 ANNUAL MEETING

The Thirty-seventh Annual Meeting of The Endocrine Society will be held in the Chalfonte-Haddon Hall Hotel, Atlantic City, New Jersey, on Thursday, Friday and Saturday, June 2, 3 and 4, 1955.

Dr. Matthew Molitch, 705 Pacific Avenue, Atlantic City, New Jersey, is in charge of the local arrangements for the meeting.

All Scientific Sessions will be held in the Chalfonte-Haddon Hall Hotel. The rooms in which each session will be held will be announced in the program and on the hotel bulletin board. The annual dinner is scheduled for Friday, June 3, at 7:30 p.m., preceded by cocktails at 6:30 p.m.

All members are urged to make hotel reservations immediately, as the hotels expect to be filled to capacity. Correspond directly with Chalfonte-Haddon Hall, advising time of arrival and departure. Make your reservations now and avoid disappointment.

THE 1955 AWARDS AND FELLOWSHIPS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers;

1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship was established in 1947. It is designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. The Fellowship is awarded on alternate years (1955, 1957, etc.) and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst, McKenna and Harrison Fellowship may be made by any member of the Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than November 1 each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by November 1 each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

The American Goiter Association

THE 1955 ANNUAL MEETING

The next annual meeting of the American Goiter Association will be held in Oklahoma City, Oklahoma, at the Skirvin Hotel, April 28, 29 and 30, 1955.

1955 LAURENTIAN HORMONE CONFERENCE

The 1955 Laurentian Hormone Conference will be held at Estes Park, Colorado, during the period September 11–16, 1955. This Western meeting has been instituted particularly to enable attendance at the meeting by qualified investigators who have found it difficult to attend the conferences at an Eastern site.

In view of a need for detailed advance arrangement, interested investigators should make application to the Committee on Arrangements of The Laurentian Hormone Conference, 222 Maple Avenue, Shrewsbury, Massachusetts, at an early date and in any event no later than May 6, 1955. Attendance at the Conference is limited by the accommodations available. A Conference rate of $12.00 per person per day is extended to all invited participants. Final announcement of the Conference program will be made early in 1955.

AMERICAN GERIATRICS SOCIETY

The 1955 Annual Meeting of the American Geriatrics Society will be held in the Hotel Roosevelt, New York City, on *April 21 and 22, 1955.* Hotel reservations should be made through Mr. A. G. Schellenberg, Reservations Manager of the Hotel Roosevelt.

The program will cover many aspects of geriatric medicine, and there will be several panel discussions on subjects of current interest.

For further information about the program, write to the Secretary of the American Geriatrics Society—Dr. Malford W. Thewlis, 25 Mechanic Street, Wakefield, R. I.

EXPERIMENTAL RESEARCH INTO PROBLEMS OF AGEING

The trustees of the *Ciba Foundation* for the Promotion of International Co-operation in Medical and Chemical Research, 41 Portland Place, Lon-

don, W.1, wishing to encourage well-conceived research relevant to basic problems of ageing, invite candidates to submit work in the field for

Awards for 1954–55.

Details of the conditions may be obtained on application to the undersigned, but in general candidates should note:

(*a*) Five awards, of an average value of £300 each, are available for the period 1954–1955. The announcement of awards will be made in July 1955.

(*b*) Entries must be received by the undersigned not later than 28th February, 1955.

(*c*) Entries will be judged by an independent international panel of distinguished scientists who will advise the Executive Council of the Foundation on their findings and will also have power to recommend variation in the size and number of the awards according to the standard of entries. The decisions of the Executive Council will be final.

(*d*) In making the awards preference will be given to younger workers.

(*e*) The work submitted should be unpublished (but may be under consideration for publication) at the closing date for entries.

(*f*) The papers may be in the candidate's own language, but a summary in English not exceeding 500 words must be attached.

(g) Where there is one or more co-author, the name of the leading author should be indicated; it is to him that the award will normally be made, and it will be left to his discretion to share this award appropriately with his co-authors.

G. E. W. WOLSTENHOLME,
Director and Secretary to the Executive Council

BOOKS RECEIVED

American Lectures in Endocrinology. Edited by Willard O. Thompson, M.D. Published by Charles C Thomas, Publisher, Springfield, Illinois.

Diabetes Mellitus. Objectives and Methods of Treatment. By HENRY T. RICKETTS, M.D., Professor of Medicine, University of Chicago, The School of Medicine; 131 pages; 6 figures; 7 tables; diets; 1955. Price $3.25.

Hypoglycemia and the Hypoglycemic Syndrome. By A. J. KAUVAR, M.D., Assistant Clinical Professor of Medicine, University of Colorado School of Medicine, and MARTIN G. GOLDNER, M.D., Clinical Associate Professor of Medicine, State University of New York, Medical Center at New York; 75 pages; 7 figures; 1 plate; 1954.

Hypothyroidism. An Essay on Modern Medicine. By PAUL STARR, M.D., Professor of Medicine & Chairman of the Department of Medicine, University of Southern California, School of Medicine, Los Angeles, California; 140 pages; 17 tables; 25 figures; 1954. Price $3.75.

The Management of Endocrine Disorders of Menstruation and Fertility. By GEORGEANNA SEEGAR JONES, M.D., Assistant Professor of Gynecology, The Johns Hopkins University; Gynecologist, The Johns Hopkins Hospital, Baltimore, Maryland; 208 pages; 37 figures; extensive bibliography; 1954. Price $5.75.

American Lectures in Abdominal Viscera. Edited by Lester R. Dragstedt, M.D. Published by Charles C Thomas, Publisher, Springfield, Illinois.

Surgery of the Adrenal Glands. By WILLIAM WALLACE SCOTT, M.D., Professor of Urology, The Johns Hopkins University School of Medicine, Urologist-in-Charge, the Johns Hopkins Hospital, Baltimore, Md., and PERRY B. HUDSON, M.D., Assistant Professor of Urology, Columbia University College of Physicians & Surgeons, Assistant Urologist, Presbyterian Hospital, New York, N. Y.; 164 pages; 35 illustrations; 1954. Price $3.50.

Hugh Roy Cullen. A Story of American Opportunity. By ED KILMAN and THEON WRIGHT; 376 pages; 1954. New York, Prentice-Hall, Inc. Price $4.00.

Le syndrome hypothalamique. Synthèse endocrinienne métabolique, végétative et psychique. Par JEAN WEILL, Médecin des Hôpitaux de Paris, et J. BERNFELD, Médecin attaché à l'Hôpital Bretonneau; préface du Professeur Rémy Collin; 260 pages: 24 figures; bibliographie; 1954. Masson et C^ie^, Éditeurs, Paris. 1.600 fr.

Medical Uses of Cortisone. Edited by FRANCIS D. W. LUKENS, M.D., Professor of Medicine, University of Pennsylvania School of Medicine; Director, The George S. Cox Medical Research Institute, University of Pennsylvania, Philadelphia, Pa. Written by 29 noted authorities; 15 chapters; 534 pages; charts and diagrams; extensively indexed; 1954. Garden City, N. Y., The Blakiston Company, Inc. Price $7.50.

Roberta Cowell's Story. An Autobiography. British Book Centre, Inc., New York, N. Y.; 208 pages; 1954. Price $3.00.

Studies in Medicine. Presented to Aleksander Rudolf Klossner on his sixtieth birthday; Supplementum 5 to Volume 43, Annales Chirurgiae et Gynaecologiae Fenniae, Helsinki, Finland. In English; tables; illustrations; 543 pages; 1954. Copenhagen K, Denmark, Ejnar Munksgaard, Ltd., International Bookseller & Publisher.

Transactions of the Josiah Macy, Jr. Foundation Conferences. Publications Sales Office, P.O. Box 575, Packanack Lake, New Jersey.

Administrative Medicine. Second Conference, December 8, 9 and 10, 1953, Princeton, New Jersey. Edited by GEORGE S. STEVENSON, M.D., National and International Consultant, National Association for Mental Health, New York; 164 pages; 1954. Price $3.00.

Liver Injury. Twelfth Conference, September 21, 22 and 23, 1953, Princeton, New Jersey. Edited by F. W. HOFFBAUER, M.D., Associate Professor, Department of Medicine, University of Minnesota Hospitals, Minneapolis, Minn.; 231 pages; 1954. Price $4.25.

Renal Function. Fifth Conference, October 14, 15 and 16, 1953, Princeton, New Jersey. Edited by STANLEY E. BRADLEY, M.D., Associate Professor of Medicine, Columbia University College of Physicians & Surgeons, New York, N. Y.; 218 pages; 1954. Price $3.75.